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Short-Term Effects of Cell Phone Radiation on Fertility and Testosterone Hormone in Male Rats

Cep Telefonu Radyasyonunun Erkek Sıçanlarda İnfertilite ve Testosteron Hormonu Üzerine Kısa Dönem Etkileri

Jafar FATAHI ASL[1,2]
0000-0002-3301-6055
Kiarash SHIRBANDI[1,2]
0000-0002-1055-6606
Anahita REZAIE[3]
0000-0002-7224-4341
Shahrzad RASTEGARPOUR[1]
0000-0002-0049-1212
Shamim PAHLAVANI[1]
0000-0002-1931-9607
Akram AHANGARPOUR[4]
0000-0002-9534-9699
Maryam DASTOORPUR[5]
0000-0002-4485-314X
Esrafil MANSOURI[6]
0000-0001-6997-6798

[1]Department of Medical Imaging and Radiation Sciences, Ahvaz Jundishapur University of Medical Sciences Faculty of Paramedicine, Ahvaz, Iran

[2]Cellular and Molecular Research Center, Ahvaz Jundishapur University of Medical Sciences Medical Basic Sciences Research Institute, Ahvaz, Iran

[3]Department of Physiology, University of Shahid Chamran School of Veterinary Medicine Ahvaz, Iran

[4]Department of Physiology and Physiology Research Center, Ahvaz Jundishapur University of Medical Sciences School of Medicine, Ahvaz, Iran

[5]Department of Biostatistics and Epidemiology, Ahvaz Jundishapur University of Medical Sciences School of Health, Ahvaz, Iran

[6]Department of Histology, Ahvaz Jundishapur University of Medical Sciences School of Medicine, Ahvaz, Iran

Corresponding Author
Sorumlu Yazar
Kiarash SHIRBANDI
shirbandi.k@gmail.com

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ABSTRACT

Aim: Given the increasing usage of cell phones (6.9 billion subscriptions globally) and heterogeneous reports, this study aimed to determine the cell phone effect as non-ionizing radiation on the level of testosterone hormone and sperm parameters in male rats.
Material and Methods: Twenty-five matured male Wistar rats were randomly allocated to five groups with the same body weights. Radiofrequency radiation for the exposed groups was 1 h/day call, 2 h/day call, and 50 missed calls/day in 30 days. The other two groups were control (out of any radiation) and positive control (exposed to γ-radiation) groups. Sperm parameters (motility, morphology, viability, counting), histopathology, and serum level of testosterone were measured and analyzed.
Results: According to the results, the sperm viability significantly decreased compared to the control group ($p<0.001$). Also, the findings revealed that the sperm motility in all groups except missed call group ($p=0.475$). For sperm count and morphology only in Group C (2 h/day call) and Group D (positive control), there were significant reductions compared to the control group ($p<0.001$). The level of testosterone was not statistically significantly different between the groups ($p=0.451$).
Conclusion: This study suggests that cell phone hazard to infertility was mild to moderate, and cell phone usage might have long-term effects on infertility. However, the cell phone cannot significantly affect the serum testosterone level.
Keywords: Cell phone; infertility; semen; testosterone; histomorphometry.

ÖZ

Amaç: Cep telefonlarının artan kullanımı (küresel olarak 6,9 milyar abonelik) ve heterojen raporlar göz önüne alındığında, bu çalışmada, iyonlaştırıcı olmayan radyasyon olarak cep telefonu erkek sıçanlarda testosteron hormonu düzeyi ve sperm parametreleri üzerine etkisinin belirlenmesi amaçlanmıştır.
Gereç ve Yöntemler: Yirmi beş olgunlaşmış erkek Wistar sıçanı, aynı vücut ağırlıklarına sahip olacak şekilde rastgele olarak beş gruba ayrıldı. Maruz kalan gruplar için radyofrekans radyasyonu 30 gün içinde günde 1 saat görüşme, günde 2 saat görüşme ve günde 50 cevapsız çağrı şeklindeydi. Diğer iki grup ise kontrol (hiçbir radyasyon uygulanmayan) ve pozitif kontrol (γ-radyasyona maruz kalan) grupları idi. Sperm parametreleri (hareketlilik, morfoloji, canlılık, sayım), histopatoloji ve serum testosteron düzeyi ölçüldü ve analiz edildi.
Bulgular: Sonuçlara göre sperm canlılığı kontrol grubuna göre anlamlı olarak azaldı ($p<0,001$). Ayrıca bulgular cevapsız çağrı grubu dışındaki tüm gruplarda sperm hareketliliğinin olduğunu ortaya koydu ($p=0,475$). Sadece Grup C'de (günde 2 saat görüşme) ve Grup D'de (pozitif kontrol) sperm sayısı ve morfolojisi açısından kontrol grubuna kıyasla anlamlı şekilde azalma vardı ($p<0,001$). Testosteron düzeyi ise gruplar arasında istatistiksel olarak anlamlı şekilde farklı değildi ($p=0,451$).
Sonuç: Bu çalışma, cep telefonunun infertilite tehlikesinin hafif ila orta düzeyde olduğunu ve cep telefonu kullanımının infertilite üzerinde uzun vadeli etkileri olabileceğini düşündürmektedir. Bununla birlikte, cep telefonu serum testosteron seviyesini ise önemli ölçüde etkileyememektedir.
Anahtar kelimeler: Cep telefonu; infertilite; semen; testosteron; histomorfometri.

INTRODUCTION

Radiofrequency electromagnetic waves (RF-EMW) in optical waves in a vacuum or matter are released (1). These waves include electric and magnetic fields divided into the frequency range of radiofrequency (RF), microwave (MW), infrared, visible light, X-rays, and gamma rays (2). Cell phones are emitting RF-EMW, which between antennas and base stations, is transmitted (3). The frequency of these devices lies within the range of 450 to 3,800 megahertz (MHz), (4,5) that is part of non-ionizing radiation (NIR) (6).

According to statistics, 20-35% of males suffer infertility, a global problem (7). Reproduction in vertebrates requires coordination between the glands of the hypothalamus, pituitary, and gonads (8,9). Among the hormones that establish reproductive coordination, the luteinizing hormones (LH) with follicular stimulatory hormones (FSH) are realized by the pituitary gland (10).

FSH stimulates Sertoli cells in the spermatozoa to produce mature sperm (11). On the other hand, LH induces the synthesis of testosterone in testicular lining cells (12). Secondary marital characteristics, anabolism, and libido by testosterone are both generated, which also causes the hypothalamus-pituitary to regulate LH secretion (13).

Identification of the biological effects of cell phones due to NIR doesn't have enough energy to dislodge electrons, complex and controversial (14). Indeed, it may produce different biological effects in irradiated molecules in terms of intensity and frequency of radiation (15,16).

The increased usage of cell phones (6.9 billion subscriptions globally) and heterogeneous reports (17), which in recent years have been devastating about the damaging effects of these waves on different growth processes, have raised concerns about the harmful cell phone effects radiations on human health. This study aimed to determine the cell phone effect as NIR on the level of testosterone hormone and sperm parameters (motility, morphology, viability, counting) in male rats.

MATERIAL AND METHODS

Study Design

According to the Animal Research Reporting of in vivo Experiments (ARRIVE) guidelines checklist (18), the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (AJUMS) approved the protocol of this study under the number of IR.AJUMS.REC.1396.255. The duration of the study was 30 days.

Animals

Twenty-five mature male Wistar rats, with the same body weights, were prepared from the animal house center at AJUMS. The rats were housed in steel cages and maintained in a ventilated room at 25±3 °C, exposed to 12 hours light and 12 hours darkness. They were given free access to water and fed a commercial diet. Once acclimatized for 2 weeks, the animals were simply randomization based on a single sequence allocated to Group A (control; n=5), Group B (1 h/day call, n=5), Group C (2 h/day call, n=5), Group D (positive control, n=5), and Group E (50 missed calls/day, n=5).

The National Institutes of Health Guide conducted the investigation. The Institutional Review Board of AJUMS approved it, and every effort to minimize both the number of animals used and their suffering was made.

Sample Size

According to the animals randomized for each experimental group 'National Centre for the Replacement, Refinement, and Reduction of Animals in Research' (NC3RS)', the sample size was calculated with the formula 'Resource Equation' N=(E+T)/T (where 10≤E≤20, N: the number of animals per treated group, E: represents the degrees of freedom of the ANOVA) was chosen 5 rats for each group (19).

Exposure System

A cell phone simulation Mobile Telecom GSM signal (Bionic Mobin mobile frequency simulator, Iran) 900 MHz RF-EMW generating was used. The electric field density was set at 0.1 W/kg of the whole-body mean specific absorption rate (SAR). The single antenna of the simulation was confined and exposed to 900 MHz RF exposure emitted (Figure 1). RF radiation for exposed groups was 1 h, 2 h, and 50 missed calls in 30 days (seven days a week). The distance from each antenna to the head of the rats was 1 cm (20). 1-mm aluminum metals entirely covered the room walls for protection from possible outside telemetry exposure.

Other Groups

The control group was placed in a different room with the same temperature and condition (25±3 °C) but out of any radiation. Meanwhile, the positive control group was exposed to 6 Gy γ-radiation with a 1.9 Gy/min dose rate (Cobalt source, Elekta, England) to induce oxidative stress in the testis and cause permanent infertility in the rats (21).

Sperm Parameters

Animals were anesthetized with ketamine xylazine at the end of the experiment, also took blood directly from the heart. According to the World Health Organization (WHO) guideline (22), the cauda of the left epididymis was separated, and sperm was analyzed. Sperm motility was divided into four categories (1. fast progressive, 2. slow progressive, 3. non-progressive, 4. non-motile) in ten microscopic fields and were shown as the motility percentage in every sample. For analyzing sperm morphology used the Papanicolaou staining method. The sperm percentage with normal morphology was then determined. For identifying sperm viability, drop sperm mixed with a small eosin drop B (0.5% in saline) was set on a slide and analyzed at ×400 magnification. Live sperm does not absorb color, with the head of a dead sperm absorbing eosin and becoming red. In each fall, 100 sperms

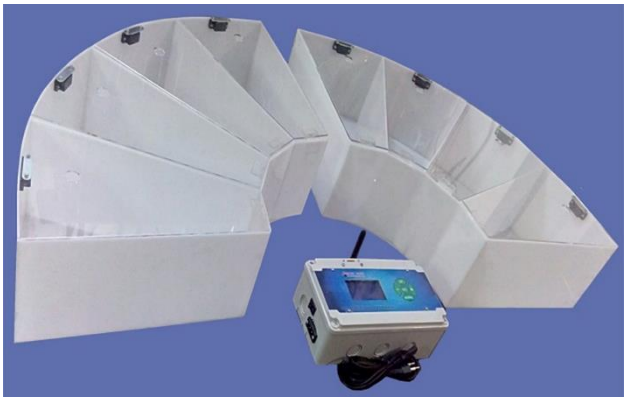

Figure 1. The single antenna of the simulation is confined in a Plexiglas carousel and exposed to 900 MHz RF

were counted, and the viable sperm percentage reported. To count sperms, used a Neubauer hemocytometer. The sperm count was calculated in one ml.

Testosterone Test

ELISA kits (monobind, USA, California) were used to measure the concentrations of serum testosterone. Intra-assay precision (precision within an assay) was used with the percent coefficient of variation (CV%) <15% for Testosterone; Inter-assay accuracy (accuracy between assays): CV% <15% for Testosterone. Finally, all homogenates were centrifuged (5,000 × g, 5 min, 4 °C), and the supernatants were stored at -80°C until measurement of Testosterone (23).

Histopathologic Analysis of the Testis

For pathologic examination, the rats' right testis was placed in 10% Bouin solution for 24 h. Following fixation, the pieces were subjected to standard histologic tissue preparation, dehydration, and paraffin embedding. With a microtome, paraffin blocks were cut to a thickness of 5 m, and the slices were stained with Hematoxylin and Eosin (H&E). They were then examined under a light microscope by the groups. The obtained images were measured in all groups equally according to the Modified Johnson scoring system (from 1 to 10), and the results were analyzed (24).

Statistical Analysis

The data showed descriptive statistics, including mean, standard deviation (SD) when a parametric test is used, and as median (interquartile range) [min-max] when using a non-parametric test. Shapiro-Wilk test used to examine normality and Levene test for homogeneity of variances. One-way ANOVA with the Tukey post hoc test or equivalent to the Kruskal-Wallis with Dunn post hoc test were used. Statistical analysis were done by Statistical Package for the Social Sciences (SPSS) v.26 software and $p<0.05$ was evaluated as statistically significant.

RESULTS

Epididymal Sperm Characteristics

Characteristics of epididymal sperm and the effects of cell phone radiation (900 MHz) on epididymal sperm were given in Table 1. According to the results, the sperm viability with 2 h/day call significantly decreased compared to the control group ($p<0.001$). However, there was no significant difference in sperm motility when compared between groups ($p=0.475$). The decrease in sperm count and morphology reductions were significant in Group C (2 h/day call) compared with the control group ($p<0.001$). Also, the weight of the left testis and left epididymis in Group B (1 h/day call) was considerably lesser than the control group ($p<0.001$).

Testosterone Levels

The testosterone levels were as follows and there was no significant difference between groups ($p=0.451$): Control group: 1.74±1.02 (2.38) [0.39-2.95], 1 h/day group: 2.33±1.41 (2.03) [0.71-4.61], 2 h/day group: 1.65±0.8 (1.68) [0.88-2.39], positive control group: 1.26±0.98 (1.64) [0.16-2.51], and 50 missed calls/day group: 1.09±0.92 (0.86) [0.23-2.44] ng/mL (Figure 2).

Histopathologic Results

Histopathological study of testes in Group A (control) showed a typical structure where seminiferous tubules were well preserved (Figure 3A). Microscopic examination of testes in Groups B (1 h/day call) and E (50 missed calls/day) revealed mild lesions (Figure 3B and 3E) while there were severe lesions in Groups D (positive control) and C (2 h/day call). They were as follows, arrest of spermatogenesis in some seminiferous tubules, interstitial edema, and undulation of basement membranes. Interstitial edema was characterized by free spaces or soft eosinophilic materials between seminiferous tubules (Figure 3D and 3C). Also, tubules were reduced and had a wavy basement membrane (Figure 3D).

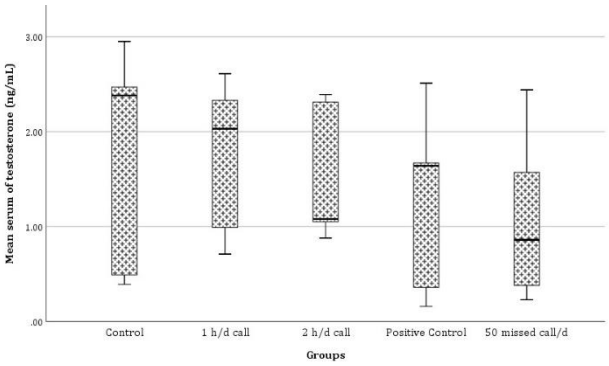

Figure 2. Serum level of testosterone in groups

Table 1. Comparison of sperm parameters between groups

	Control	1 h/day call	2 h/day call	Positive Control (γ-radiation)	50 missed calls/day	p
Sperm Viability (%)	90.52±1.56 (90) [89-93]	81.84±1.75 (82.3) [79-83.4]	69.32±3.17 (68.5) [66-74.5]	35.94±3.07 (34.5) [32.4-39.6]	82.02±1.51 (82) [80.1-83.6]	**<0.001**[*] **<0.001**[∫]
Sperm Motility (%)	52.30±3.55 (51.43) [48-56.78]	42.08±3.77 (41.5) [37.8-47.9]	37.76±4.35 (39.8) [30.23-40.98]	0.13±0.10 (0.1) [0.01-0.32]	48.14±5.63 (46.9) [42.7-57]	0.475[*] 0.812[∫]
Sperm Count ($\times 10^6$)	60.82±2.34 (61.7) [57-62.9]	58.19±6.42 (57) [50.1-68]	42.82±2.9 (43.2) [38.9-46]	0.56±0.07 (0.51) [0.43-0.67]	58.38±4.7 (59.1) [51-64]	**<0.001**[*] **<0.001**[∫]
Normal Morphology (%)	86.26±2.42 (86) [84-90]	85.91±3.17 (85) [81.3-90]	71.68±1.90 (71) [70.1-75]	23.6±3.36 (23) [20-28]	84.8±2.77 (84) [82-89]	**<0.001*** **<0.001**[∫]
Left Testis (g)	1.48±0.20 (1.45) [1.38-1.51]	1.42±0.04 (1.39) [1.35-1.46]	1.45±0.37 (1.46) [1.41-1.48]	0.92±0.07 (0.91) [0.89-0.94]	1.51±0.13 (1.52) [1.48-1.53]	**<0.001**[¥] **<0.001**[Θ]
Left Epididymis (g)	0.51±0.09 (0.52) [0.50-0.53]	0.49±0.12 (0.48) [0.45-0.50]	0.50±0.25 (0.51) [0.49-0.55]	0.36±0.01 (0.38) [0.34-0.40]	0.51±0.28 (0.53) [0.50-0.55]	**<0.001**[¥] **<0.001**[Θ]

[*]: Kruskal-Wallis test, [∫]: Dunn test, [¥]: One-Way ANOVA, [Θ]: Tukey test, descriptive statistics were given as mean±standard deviation (median) [minimum-maximum]

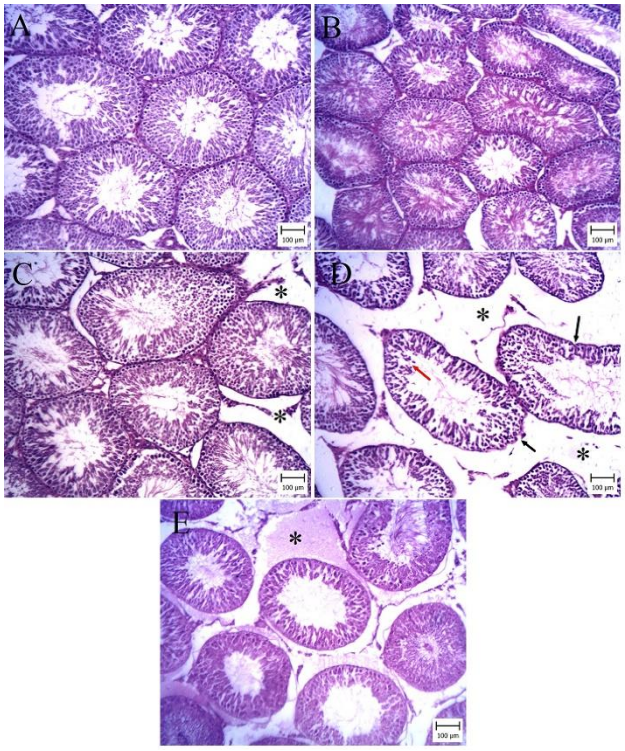

Figure 3. Photomicrograph of rat testes stained with H&E. **A)** Control, **B)** 1 h/day call, **C)** 2 h/day call, **D)** positive control, **E)** 50 missed calls/day. Note the interstitial edema (Asterisks) in (C), (D), and (E). Also, undulated basement membrane (Black arrows) and arrest of spermatogenesis (Red arrow) are seen in (D)

DISCUSSION

NIR does not have enough energy to move electrons (14). Radiation to forecast any biological effect of NIR, free radicals should be proved, oxidative stress, and DNA damage pathway. Our study indicated that cell phone radiation in the short term and sparse usage could not affect sperm motility. However, the sperm count and the average sperm viability were significantly decreased in Group C (2 h/day call) compared with the control group. Histometric indications of testicular reduced considerably than the control group, including the height of the epithelial cells of the spermatozoa, as well as the number and diameter of the Leydig cell nucleus and some of the anatomical parameters, including the size of the medium and the testicular weight in the group C (2 h/day call), and Group D (positive control).

Regarding humans studies, cell phone radiation's effects were harmful to sperm parameters (25-29). Cell phone use negatively affects sperm quality in men by reducing the semen volume (26,28), sperm count (25,27), motility, viability (25), regular morphology (25,29), and sperm DNA fragmentation could represent the only parameter significantly (26). Similarly, evidence shows that cell phone radiation can change sperm parameters (19,20,26-30). According to these studies, cell phone radiation negatively affects morphologic and histological changes (31,32). RF-EMF increased oxidative stress due to the heat and other stress-related (33), decreased gonadotropic hormonal (27), increase in apoptosis, reductions weight of the testes, negative impact on testicular architecture and enzymatic activity (34), and could negatively affect male fertility (19,20,29) by reducing sperm viability and motility (32). Consequently, our results follow other studies that considerably decreased the sperm count, the weight of the left testis and left epididymis, and the average sperm viability compared with the control group. However, Lewis RC et al. (35) and Nakatani-Enomoto S et al. (36) suggested that there was no evidence of a connection between cell phone usage and the quality of normal human spermatozoa or sperm.

Nonetheless, some studies showed no evidence for connecting cell phone use and abnormalities (37-42). Based on these studies, short-time exposure does not offer a significant risk factor for rat reproductive functions and the number of sperm in the testis and epididymis.

At the same time, evidence suggests that cell phone radiation influences infertility in men (33,43-48). These in vitro studies reported that cell phone transpiration has a harmful sperm acrosin activity, enhances mitochondrial reactive oxygen species generation (49), leads to oxidative stress (50), decreases sperm motility (33,38,40,51) and vitality (52). However, few in vitro studies show cell phones may increase safety exposure in their sample (44,45). Nevertheless, the current study showed a significant decrease in the sperm count and the average sperm viability; the mean percentage of motility was not significantly compared between groups.

Our study showed that cell phone radiation does not affect the testosterone hormone. Also, Jin YB et al. (51) suggested that RF did not affect serum levels in testosterone. However, some studies reported a decrease in the serum testosterone level with increasing the period of exposure (53-55). The most important argument for this is reducing the number and diameter of the Leydig cell nucleus and decreasing the interstitial testicular tissue as the site of synthesis and secretion of testosterone in the Leydig cell cytoplasm in the testicular tissue.

CONCLUSION

The results of this study suggest that cell phone hazard to infertility was mild to moderate, and usage of the cell phone might cause long-term effects on infertility. Also, the cell phone cannot significantly influence the serum testosterone level.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (16.05.2017, IR.AJUMS.REC.1396.255).

Conflict of Interest: None declared by the authors.

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Author Contributions: Idea/Concept: KS; Design: JFA; Data Collection/Processing: AA, EM; Analysis/Interpretation: AR, MD; Literature Review: SR, SP; Drafting/Writing: JFA, KS; Critical Review: JFA, KS.

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