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ORIGINAL RESEARCH

Effects of cell phone waves on testes – a biochemical and histological experimental study

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Background: The wide use of cell phones in recent years has raised many questions regarding the safety of prolonged exposure to its associated high-frequency electromagnetic radiation (EMR), especially for testes which are vulnerable to its hazards. This study aims to experimentally study the possible hazardous effects of cell phones' associated EMR on testicular functions and structures.

Materials and methods: A total of 50 adult Wistar albino rats were divided into a control group (10 rats in group I) and EMR-exposed rats for three and six months (groups II and III respectively, 20 rats each). By the third and sixth months levels of serum testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin were evaluated in comparison to the control rats. Sacrificed rats' testes were examined using light microscopy and electron microscopy.

Results: Testosterone levels were significantly lower in groups II and III compared to group I ($p < 0.001$). FSH, LH, and prolactin didn't show a statistically significant difference among the three groups ($p > 0.05$). Light microscopy showed progressive degenerative changes in different seminiferous tubules with intact interstitial collagen bundles, except around degenerated tubules in groups II and III. Electron micrographs suggested Leydig cells to be the most affected structure with significant degenerative changes.

Conclusion: Under the circumstances of this experimental study in rats, prolonged close exposure to cell phones seems to have obvious deleterious effects on testicular tissues and functions. This is supported by serum testosterone level reduction and histopathological degenerative changes in testicular seminiferous tubules and Leydig cells.

Keywords: cellular phone waves, cell phones, testes, male infertility, testicular dysfunctions

Introduction

In the modern era, cell phones have evolved from simple communication devices to multipurpose tools used to surf the internet, provide GPS navigation, as well as video conferencing, gaming, etc.[1] The radiofrequency (RF) EMR emitted from cell phones as well as Wi-Fi devices is described as non-ionizing radiation. Despite its low output power, the persistent use of these devices and their proximity to users' bodies could theoretically affect the health of different tissues.

There are many debatable reports linking cell phone RF exposure to human tissues' structural and functional changes.[2–7] The adverse effects of EMR on the heart, blood pressure, brain, and endocrine system were widely reported.[7]

Regarding the male reproductive system, the issue is still debatable. Early reports didn't prove any hazardous effects of cell phone-emitted EMR, either on testicular functions or structures.[8,9] However, others showed EMR and cell phones could decrease the semen quality in animal models as well as in men, mainly sperm count, motility, viability, and normal morphology.[10] These effects were found to be directly related to the duration of daily cell phone use.[10,11]

Due to the worldwide concern about its possible health hazards, the current research was designed to experimentally evaluate the testicular biochemical and structural (light and electron microscopic) changes induced by cell phones' emitted RF waves.

Materials and methods

Study protocol and materials

This experimental study was performed under conventional laboratory conditions at the animal house of the Histology Department, Faculty of Medicine, at Tanta University in Egypt. A total of 50 adult male Wistar albino rats with an average body weight of 200 ± 10 g and aged 80–90 days were included. All animals were housed in cages (5 animals per cage) under standard conditions of 23–25 °C with 42 ± 5% relative humidity and a 12/12 hour light-dark cycle. The rats had unlimited access to food and water, and all moral principles regarding the use and treatment of animals were taken into consideration. This study was approved by the ethical committee of the Faculty of Medicine at Tanta University (approval reference 32686/11/18).

EMR was produced by cell phones with a network band of (GSM) 900 MHz. A cell phone was situated 3 cm below the centre of the cages which measured 30 × 40 × 40 cm. The maximal distance from the phone to the floor corners was around 28 cm.

During the one-hour exposure to EMR in groups II and III, all cages of the three groups were covered with aluminium foil to limit the EMR field inside the cages in groups II and III and keep a similar dark surrounding environment in the control animals. During daily EMR exposure, the cell phone was turned on and kept on call mode for one hour, then turned to standby mode for 10 hours before it was removed till the next day.

Wistar rats were randomly divided into three groups according to cell phone EMR exposure and its duration:

- Group I (control group): consisted of 10 rats living away from the nearby source of EMR.
- Group II: consisted of 20 rats exposed to EMR fields for one hour daily for three months.
- Group III: consisted of 20 rats exposed to EMR fields for one hour daily for six months.

Rats in groups II and III were sacrificed by the end of the third and sixth months respectively. At this time, rats were anaesthetised using ketamine HCl (50–100 mg/kg), administered intraperitoneally, and subjected to the following:

- Hormonal assays: 2–3 ml blood was collected from the aorta and tested for the levels of testosterone, FSH, LH, and prolactin using the Roche Cobas E411 analyser, which is a fully automated analyser that uses patented electrochemiluminescence technology for immunoassay analysis.
- Histopathological examination: a median abdominal incision was performed to expose the two testes (Figure 1). They were carefully dissected and divided into two parts. One part was fixed in Bouin's solution for light microscopic examination. The other part was fixed in a paraformaldehyde solution for electron microscopic examination.

Preparation of specimens for light microscopy examination

After fixation, the specimens were dehydrated in ascending grades of alcohol cleared in the two changes of xylene to be embedded in paraffin. Finally, sections 5 μm thick were cut by microtome to be stained by haematoxylin and eosin (H&E) stain for general histological features and Verhoeff van Gieson's (VVG) stain for evaluating collagen and elastic fibres.

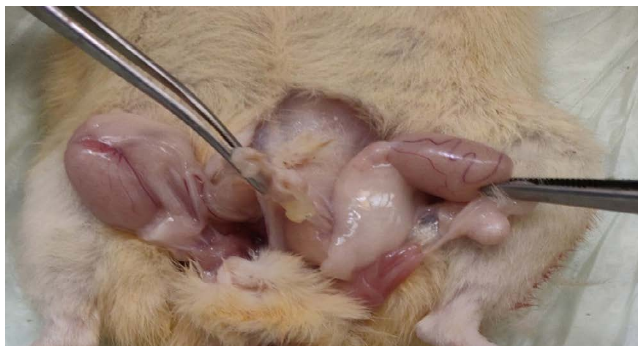

Figure 1: A median abdominal incision with two exposed testes

Preparation of specimens for transmission electron examination

For electron microscopy, 1 mm^3 from testes was prepared and fixed in 2.5% glutaraldehyde solution with 5% phosphate buffer (pH 7.4) at 4 °C for two hours. Thereafter washed three times with 0.1 M phosphate buffer and post-fixed in 1% osmium tetroxide in a phosphate buffer (pH 7.4) for two hours. Dehydration was carried out through a graded series of ethanol and embedded in an Epon-Araldite mixture. After polymerisation overnight at 70 °C, semi-thin sections (1 μm) were cut to localise the area for transmission electron microscopic examination and photographing. After that, ultrathin sections (60–80 nm) were cut using an ultra-microtome and were stained with uranyl acetate and lead citrate to be examined and photographed using a Jeol-JEM-100SX electron microscope at the Electron Microscopic unit, Histology Department, Faculty of Medicine, Tanta University.

Statistical analysis of the data

Data regarding hormonal assays were organised and presented as mean ± standard deviation (SD). Multiple measures ANOVA and Tukey post-tests were used to compare results among the three groups.

Results

In the present study, no mortality was recorded during the study period. By the end of the study, all rats were euthanised.

Hormonal assays findings

No statistically significant difference was noted between the three groups regarding FSH, LH, and prolactin levels. Only testosterone showed significantly lower values that were noticed by the third month (group II) and more decreased by the sixth month (group III) (Table I). Statistics were calculated using the F-test.

Histological findings after H&E staining in group I

Testicular tissues in group I showed intact seminiferous tubules with intact basement membranes covering normal germinal epithelium (Figure 2).

Table I: Hormonal assay results in the three groups

		Group I	Group II	Group III	*p*-value
FSH (mIU/mL)	Range	2.65–3.1	2.58–3.05	2.57–3.12	0.41
	Mean ± SD	2.81 ± 0.17	2.76 ± 0.15	2.73 ± 0.15	
LH (mIU/mL)	Range	3.35–4.39	3.31–4.46	3.16–4.54	0.65
	Mean ± SD	3.76 ± 0.30	3.69 ± 0.32	3.64 ± 0.37	
Prolactin (ng/mL)	Range	28.1–61.2	27.3–60.5	25.6–58.6	0.66
	Mean ± SD	47.43 ± 12.4	44.19 ± 10.95	43.61 ± 10.38	
Testosterone (ng/mL)	Range	3.27–3.81	2–2.18	0.8–1.73	$p < 0.001$
	Mean ± SD	3.60 ± 0.20	2.07 ± 0.07*	1.16 ± 0.26*□	

*Statistically significant compared to group I ($p < 0.001$)
□Statistically significant compared to groups I and II ($p < 0.001$)

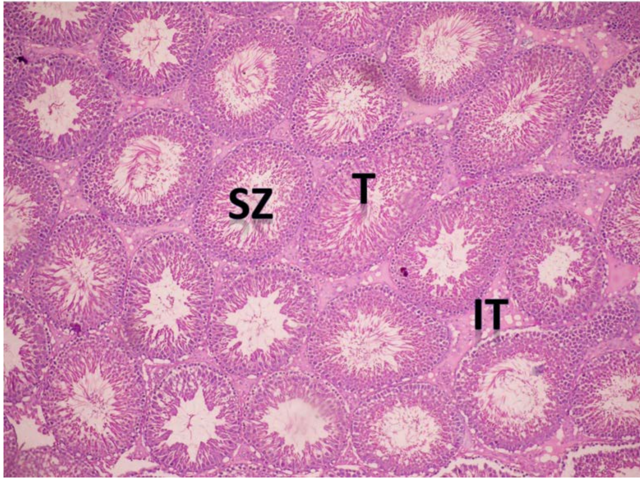

Figure 2: Photomicrograph of testes of the control group shows adjacent normal oval to rounded seminiferous tubules (T); spermatozoa (SZ) are filling the lumina of the tubules; interstitial tissue (IT) can be seen between the tubules (H&E X 200)

Histological findings after H&E staining in group II

The three-month exposed group displayed marked degenerative changes in different seminiferous tubules which became widely separated from each other with focal separation of the basement membrane from the overlying germinal epithelium. Some seminiferous tubules were devoid of sperms and lined by disconnected spermatogenic cells (Figure 3).

Histological findings after H&E staining in group III

The six-month exposed group revealed loss of architecture and disrupted and dilated seminiferous tubules. The spermatogenic cells of many tubules were disorganised, widely separated, necrotic or depicted complete depletion (Figure 3).

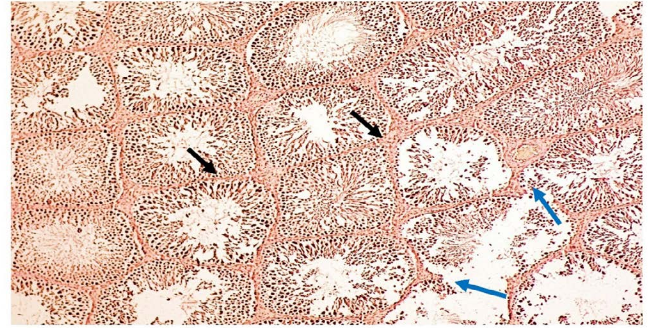

Figure 4: Photomicrographs of the testes section of group III show red-coloured collagen fibres (black arrows) in the interstitial tissue between the seminiferous tubules; note its absence around the disrupted one (blue arrows) (VVG X 200)

Histological findings after VVG staining in groups I, II, and III

There was evidence of thin red collagen fibres in the interstitial tissues between the seminiferous tubules without any differences between the studied groups I, II, and III. However, no collagen fibres were detected around the disrupted seminiferous tubules (Figure 4).

Electron microscopic histological findings in group II

The interstitial Leydig cells revealed disorganised vacuolated cytoplasm, dilated smooth endoplasmic reticulum (SER), few lipid droplets, and few mitochondria with disrupted cell membranes (Figure 5).

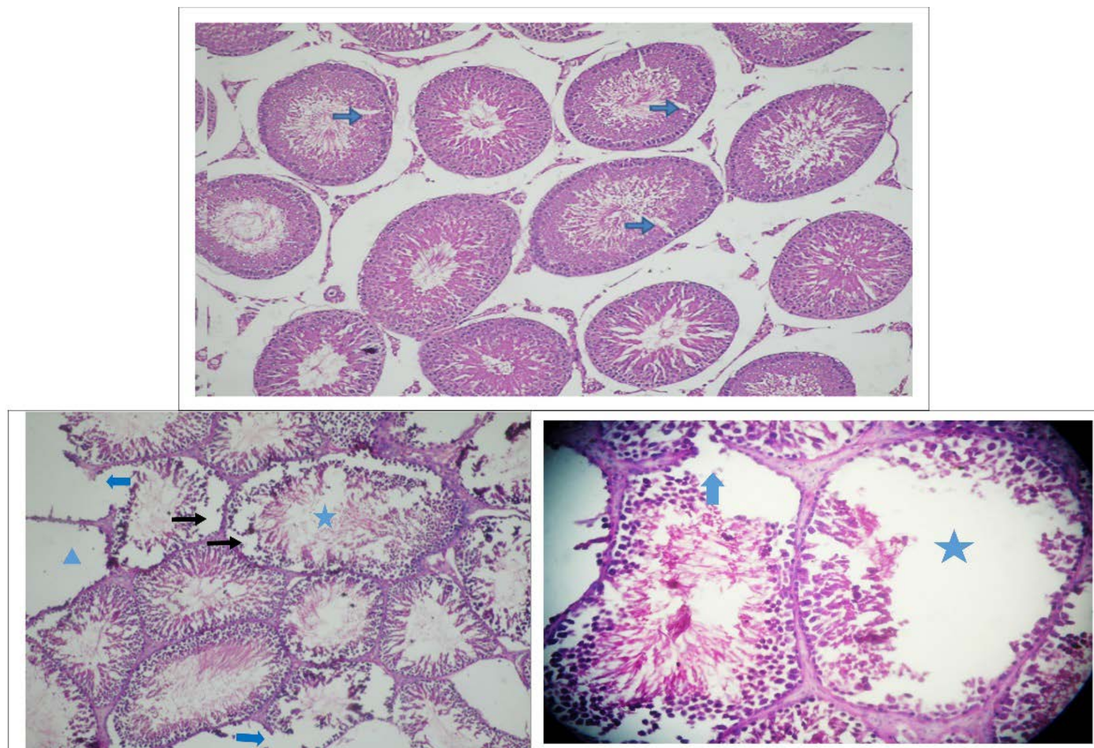

Figure 3: Photomicrograph of rat testes of groups II (upper part) and III (lower part) showing loss of architecture, disrupted (blue arrow), dilated (star), and empty seminiferous tubule (triangle) with sub-epithelial separation (black arrow); the spermatogenic cells are disorganised and widely separated, necrotic and totally absent (H&E X 200 and X 400)

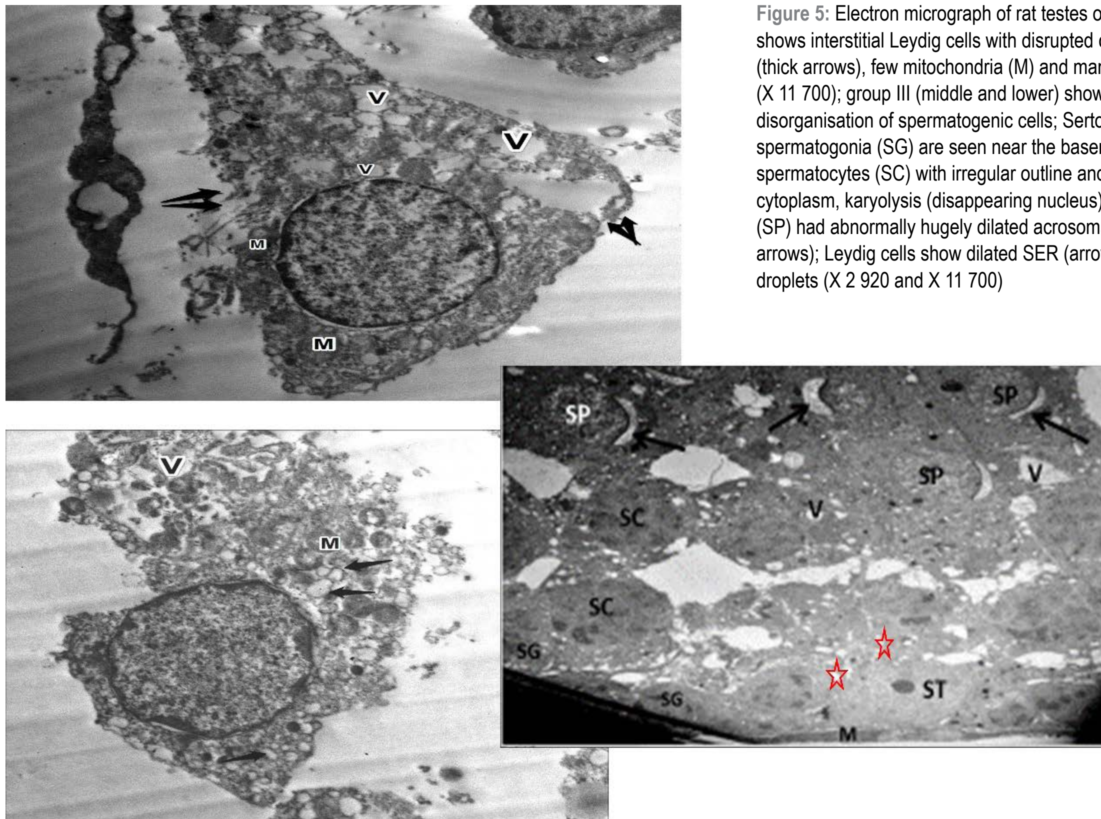

Figure 5: Electron micrograph of rat testes of group II; (upper) shows interstitial Leydig cells with disrupted cell membrane (thick arrows), few mitochondria (M) and many vacuoles (v) (X 11 700); group III (middle and lower) showed distortion and disorganisation of spermatogenic cells; Sertoli cells (ST) and spermatogonia (SG) are seen near the basement membrane; spermatocytes (SC) with irregular outline and vacuolated cytoplasm, karyolysis (disappearing nucleus) (star); spermatids (SP) had abnormally hugely dilated acrosomal vesicles (thin arrows); Leydig cells show dilated SER (arrow) and few lipid droplets (X 2 920 and X 11 700)

Electron microscopic histological findings in group III

The electron micrograph of the testes of group III showed wide separation, distortion and disorganisation of spermatogenic cells. Spermatocytes disclose irregular outlines and vacuolated cytoplasm with karyolysis (disappearing nucleus). Spermatids were seen with abnormally hugely dilated acrosomal vesicles. In addition, interstitial Leydig cells revealed the same changes seen in group II (Figure 5).

Discussion

Cell phones emit RF electromagnetic waves at a frequency ranging from 800–2 200 MHz and even higher for the 5G bands. The EMR-induced tissue changes could be attributed to either thermal or non-thermal effects.[12] Thermal effects are related to holding cell phones for long periods, especially close to the body. Meanwhile, the non-thermal effect of EMR is explained by the induction of oxidative stress through the release of reactive oxygen species (ROS).[13]

Testes are considered a very vulnerable organ to the hazardous effects of EMR. The testes have a limited ability to dissipate heat. In addition, testes have high rates of cell division with consecutive higher oxygen consumption. The higher oxidative process together with the extensive proliferation rates may result in DNA replication errors, which could increase the levels of free radicals within the tissues.[14,15] These factors were linked to a higher incidence of cell death and interruption of the germ cell cycle.[16,17] As germ cells play an important role in the hypothalamus-pituitary-gonadal (HPG) axis, it could disrupt hormonal balance.[14] Additionally, the literature-based findings of EMR-induced disruption of Leydig cells may explain why there is insufficient testosterone release in response to LH.[18]

In the current study, our results didn't find significant changes in pituitary gonadotrophic hormones (FSH and LH) or prolactin (which could affect the release of gonadotrophic hormones) in EMR-exposed rats compared to the control group. However, a significant reduction in serum testosterone of the exposed rats was noted compared to controls.

Unfortunately, the literature carries much heterogeneous data regarding EMR-induced pituitary and gonadal hormone changes. Çetkin et al.[19] concluded that pituitary and gonadal hormones (FSH, LH, and testosterone) didn't change significantly after exposing 32 male Wistar albino rats to 900 MHz cell phone radiation for two hours daily for 10 weeks. On the other hand, Azimzadeh et al.[20] showed that adult male Sprague-Dawley rats exposed to EMR had lower testosterone levels after exposure to 900 MHz EMR for two and four hours daily for 30 days. This was associated with changes in regulatory molecules of testes, such as steroidogenic acute regulatory protein (StAR), P450scc, tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-1α (IL-1α), and nerve growth factor (NGF), which regulates Leydig cell functions.[8]

Contrary to the results of the present study, Oyewopo et al.[14] found that rats exposed to EMR for two and three hours daily for 28 days had lower levels of gonadotropic hormones (FSH, LH, and testosterone) when compared to a control or one-hour exposure groups. They explained this as the harmful effect of ROS, which they had also proved to be higher in exposed groups.[14]

Although changes in gonadotrophic hormones are debatable, many studies support the decreased level of testosterone in EMR-exposed experimental animals.[18] In our study, the decreased levels of testosterone in groups II and III could be attributed to

the impairment of Leydig cells as they are considered among the most vulnerable cells to EMR. This suggestion is supported by our electron microscopic analysis of Leydig cells that were severely damaged in the exposed groups.

The decreased testosterone level in our results was associated with degenerative changes documented by the light microscopy findings. H&E stained testicular tissues showed marked degenerative changes in different seminiferous tubules; some tubules were devoid of sperms, worsening after six months when the testes revealed further loss of architecture. Regarding interstitial tissues, VVG staining of testes didn't show significant changes between the control and exposed groups. All groups demonstrated thin red collagen fibres in the interstitial tissues between the seminiferous tubules, which is considered normal.

Limited but debatable data in the literature discussed similar histological findings in experimental EMR-exposed rats' testes. Lee et al.[8] showed no significant testicular changes in rats exposed to 850 MHz EMR for 90 minutes daily for 12 weeks. Similarly, Ribeiro et al.[21] showed that male Wistar rats exposed to one-hour cell phone GSM 1 850 MHz radiation daily for 11 weeks had no deleterious effects on testicular tissues. However, Çetkin et al. showed lower testicular weight and volume in male Wistar albino rats exposed to 890–910 MHz EMR two hours daily for 10 weeks. This was associated with a higher fraction of interstitial tissues and a lower fraction of tubular tissue in the EMR-exposed groups. The mean seminiferous tubular diameter and germinal epithelium height were also decreased in EMR-exposed animals.[19]

To explain the mechanisms behind our hormonal and histological findings, we tried to apply electron microscopic evaluation. Electron microscopy sections after three months of EMR exposure showed disorganised Leydig cells with significant degenerative changes. After six months of exposure, degenerative changes were more extensive, including spermatocytes and spermatogenic cells.

Our electron microscopy findings are similar to those reported by Çelik et al.[22] Based on their study, there were no significant abnormalities in light microscopic findings. However, electron microscope analysis revealed an increase in the thickness of membrana propria and collagen fibres as well as Sertoli cells, and degenerative changes in Wistar-Kyoto male rats exposed to EMR daily for three months.[22]

Limitations in our study included the lack of seminal fluid evaluation. Testicular histological changes will finally affect sperm production and functions causing fertility issues. Further studies are still warranted to document this issue.

It was noted that our biochemical and histological findings partially agreed and partially refuted previous reports. The literature carries various debatable aspects regarding EMR-exposure effects on experimental rats' testes, which are still inconclusive and controversial.[11,23] This heterogeneity can be explained by the differences in the exposure protocols, species and race of animals, and differences in outcome evaluation parameters. However, together with our results, many reports document the possible hazards of EMR on rats' testes.[24-26] Transferring such conclusions to human subjects is still a complicated process as humans have different exposure times and proximity to cell phones' EMR.[23]

Conclusion

Under the circumstances of the current study, the use of cell phones seems to have obvious deleterious effects on male testicular structures and functions. The literature carries heterogeneous reports that support our results. Our findings are in part explained by the electron microscopic degenerative changes seen in Leydig cells from as early as three months and extended to include spermatogenic cells by six months. Although our conclusion draws attention to the possible hazardous effects of cell phones' EMR, these effects are dependent on the duration of exposure to cell phone EMR.

Conflict of interest

The authors declare no conflict of interest.

Funding source

None.

Ethical approval

This study was approved by the ethical committee of Tanta University, Faculty of Medicine, Research Ethics Committee (Ref: 32686/11/18).

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References

1. Ezhilarasan E, Dinakaran M. A review on mobile technologies: 3G, 4G and 5G. In: 2017 second international conference on recent trends and challenges in computational models (ICRTCCM). IEEE; 2017. https://doi.org/10.1109/ICRTCCM.2017.90.
2. Miller AB, Sears ME, Morgan LL, et al. Risks to health and well-being from radio-frequency radiation emitted by cell phones and other wireless devices. Front Public Health. 2019;7:223. https://doi.org/10.3389/fpubh.2019.00223.
3. Ozguner F, Altinbas A, Ozaydin M, et al. Mobile phone-induced myocardial oxidative stress: protection by a novel antioxidant agent caffeic acid phenethyl ester. Toxicol Ind Health. 2005;21(7-8):223-30. https://doi.org/10.1191/0748233705th228oa.
4. National Toxicology Program. Toxicology and carcinogenesis studies in Sprague Dawley (Hsd: Sprague Dawley SD) rats exposed to whole-body radio frequency radiation at a frequency (900 MHz) and modulations (GSM and CDMA) used by cell phones. Natl Toxicol Program Tech Rep Ser. 2018;(595). https://doi.org/10.22427/NTP-TR-595.
5. International Commission on Non-Ionizing Radiation Protection. ICNIRP note: critical evaluation of two radiofrequency electromagnetic fields animal carcinogenicity studies published in 2018. Health Phys. 2020;118(5):525-32. https://doi.org/10.1097/HP.0000000000001137.
6. National Toxicology Program. Toxicology and carcinogenesis studies in B6C3F1/N mice exposed to whole-body radio frequency radiation at a frequency (1,900 MHz) and modulations (GSM and CDMA) used by cell phones. Natl Toxicol Program Tech Rep Ser. 2018;(596). https://doi.org/10.22427/NTP-TR-596.
7. Melnick RL. Commentary on the utility of the National Toxicology Program study on cell phone radiofrequency radiation data for assessing human health risks despite unfounded criticisms aimed at minimizing the findings of adverse health effects. Environ Res. 2019;168:1-6. https://doi.org/10.1016/j.envres.2018.09.010.
8. Lee HJ, Pack JK, Kim TH, et al. The lack of histological changes of CDMA cellular phone-based radio frequency on rat testis. Bioelectromagnetics. 2010;31(7):528-34. https://doi.org/10.1002/bem.20589.

9. Dasdag S, Akdag MZ, Aksen F, et al. Whole body exposure of rats to microwaves emitted from a cell phone does not affect the testes. Bioelectromagnetics. 2003;24(3):182-8. https://doi.org/10.1002/bem.10083.
10. Agarwal A, Deepinder F, Sharma RK, Ranga G, Li J. Effect of cell phone usage on semen analysis in men attending infertility clinic: an observational study. Fertil Steril. 2008;89(1):124-8. https://doi.org/10.1016/j.fertnstert.2007.01.166.
11. La Vignera S, Condorelli RA, Vicari E, D'Agata R, Calogero AE. Effects of the exposure to mobile phones on male reproduction: a review of the literature. J Androl. 2012;33(3):350-6. https://doi.org/10.2164/jandrol.111.014373.
12. Behari J. Biological responses of mobile phone frequency exposure. Indian J Exp Biol. 2010;48(10):959-81.
13. Saliev T, Begimbetova D, Masoud AR, Matkarimov B. Biological effects of non-ionizing electromagnetic fields: two sides of a coin. Prog Biophys Mol Biol. 2019;141:25-36. https://doi.org/10.1016/j.pbiomolbio.2018.07.009.
14. Oyewopo AO, Olaniyi SK, Oyewopo CI, Jimoh AT. Radiofrequency electromagnetic radiation from cell phone causes defective testicular function in male Wistar rats. Andrologia. 2017;49(10). https://doi.org/10.1111/and.12772.
15. Almansa-Ordonez A, Bellido R, Vassena R, Barragan M, Zambelli F. Oxidative stress in reproduction: a mitochondrial perspective. Biology (Basel). 2020;9(9). https://doi.org/10.3390/biology9090269.
16. Kesari KK, Behari J. Evidence for mobile phone radiation exposure effects on reproductive pattern of male rats: role of ROS. Electromagn Biol Med. 2012;31(3):213-22. https://doi.org/10.3109/15368378.2012.700292.
17. Kao SH, Chao HT, Chen HW, et al. Increase of oxidative stress in human sperm with lower motility. Fertil Steril. 2008;89(5):1183-90. https://doi.org/10.1016/j.fertnstert.2007.05.029.
18. Maluin SM, Osman K, Jaffar FHF, Ibrahim SF. Effect of radiation emitted by wireless devices on male reproductive hormones: a systematic review. Front Physiol. 2021;12:732420. https://doi.org/10.3389/fphys.2021.732420.
19. Çetkin M, Kizilkan N, Demirel C, et al. Quantitative changes in testicular structure and function in rat exposed to mobile phone radiation. Andrologia. 2017;49(10). https://doi.org/10.1111/and.12761.
20. Azimzadeh M, Jelodar G. Alteration of testicular regulatory and functional molecules following long-time exposure to 900 MHz RFW emitted from BTS. Andrologia. 2019;51(9):e13372. https://doi.org/10.1111/and.13372.
21. Ribeiro EP, Rhoden EL, Horn MM, et al. Effects of subchronic exposure to radio frequency from a conventional cellular telephone on testicular function in adult rats. J Urol. 2007;177(1):395-9. https://doi.org/10.1016/j.juro.2006.08.083.
22. Çelik S, Aridogan IA, Izol V, et al. An evaluation of the effects of long-term cell phone use on the testes via light and electron microscope analysis. Urology. 2012;79(2):346-50. https://doi.org/10.1016/j.urology.2011.10.054.
23. Okechukwu CE. Does the use of mobile phone affect male fertility? A mini-review. J Hum Reprod Sci. 2020;13(3):174-83. https://doi.org/10.4103/jhrs.JHRS_126_19.
24. Erogul O, Oztas E, Yildirim I, et al. Effects of electromagnetic radiation from a cellular phone on human sperm motility: an *in vitro* study. Arch Med Res. 2006;37(7):840-3. https://doi.org/10.1016/j.arcmed.2006.05.003.
25. Falzone N, Huyser C, Becker P, Leszczynski D, Franken DR. The effect of pulsed 900-MHz GSM mobile phone radiation on the acrosome reaction, head morphometry and zona binding of human spermatozoa. Int J Androl. 2011;34(1):20-6. https://doi.org/10.1111/j.1365-2605.2010.01054.x.
26. Fejes I, Závaczki Z, Szöllosi J, et al. Is there a relationship between cell phone use and semen quality? Arch Androl. 2005;51(5):385-93. https://doi.org/10.1080/014850190924520.