Research in Veterinary Science 93 (2012) 1001–1005

Contents lists available at SciVerse ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Effects of exposure to electromagnetic field (1.8/0.9 GHz) on testicular function and structure in growing rats

H. Ozlem Nisbet [a,*], Cevat Nisbet [b], Aysegul Akar [c], Mesut Cevik [d], M. Onder Karayigit [e]

[a] Department of Surgery, Veterinary Faculty, University of Ondokuz Mayis, Samsun, Turkey
[b] Department of Biochemistry, Veterinary Faculty, University of Ondokuz Mayis, Samsun, Turkey
[c] Department of Biophysics, Medicine Faculty, University of Ondokuz Mayis, Samsun, Turkey
[d] Department of Reproduction and Artificial Insemination, Veterinary Faculty, University of Ondokuz Mayis, Samsun, Turkey
[e] Department of Pathology, Veterinary Faculty, University of Ondokuz Mayis, Samsun, Turkey

ARTICLE INFO

Article history:
Received 5 July 2011
Accepted 31 October 2011

Keywords:
Electromagnetic field
Testicular function
Testicular structure
Testosterone
Growing rat

ABSTRACT

The aim of our study was to evaluate the possible effects of whole-body electromagnetic field (EMF) exposure on reproduction in growing male rats. Male albino Wistar rats (2 days old) were exposed to EMF 1800 and 900 MHz for 2 h continuously per day for 90 days. Sham control was kept under similar conditions except that the field was not applied for the same period. After blood samples were collected, the animals were sacrificed 24 h after the last exposure and the tissues of interest were harvested. The mean plasma total testosterone showed similarity among the two study groups and was significantly higher than the sham control rats. The percentage of epididymal sperm motility was significantly higher in the 1800 MHz group ($P < 0.05$). The morphologically normal spermatozoa rates were higher and the tail abnormality and total percentage abnormalities were lower in the 900 MHz group ($P < 0.05$). Histopathologic parameters in the 1800 MHz group were significantly higher ($P < 0.05$). In conclusion, the present study indicated that exposure to electromagnetic wave caused an increase in testosterone level, epididymal sperm motility (forward), and normal sperm morphology of rats. As a consequences, 1800 and 900 MHz EMF could be considered to be a cause of precocious puberty in growing rats.

1. Introduction

Developments in technology and industry have facilitated human life. However, environmental pollution has occurred as a result of these developments and is causing a threat to human and animal lives. Studies related to the biologic effects of EMF include a broad spectrum of systems, from bacteria to the cell level (Rojavin and Ziskin, 1995; Atmaca et al., 1996), the chromosome and DNA level (Lai and Singh, 1995, 1997), and the nervous, endocrine, immune, cardiovascular, hematopoietic, and ocular systems (Roberts et al., 1986; Kolosova et al., 1996).

Many studies have reported that EMF can have adverse effects on reproduction. However, limited data have been published with regard to these potential adverse effects (Al-Akhras et al., 2001; Heredia-Rojas et al., 2004; Lee et al., 2004). Moreover, there have been conflicting findings regarding the alteration of spermatogenic and reproductive functions. A number of studies showed that exposure to EMF did not induce any adverse effects on the spermatogenesis and the reproductive capacity of animals and humans (Al-Akhras et al., 2001; Heredia-Rojas et al., 2004; Lee et al., 2004). In contrast, some studies conducted by other investigators showed clear damage to spermatogenesis (World Health Organization (WHO), 1987; De vita et al., 1995; Lee et al., 2004). Dasdag et al. (1999) reported that the diameter of the seminiferous tubule in the testes of rats was decreased even after a brief daily exposure at a whole-body SAR of 0.141 W/kg for a month to mobile phone radiation from commercially available GSM (890–915 MHz) phones. However, a subsequent study carried out to explore these results more thoroughly found that longer daily exposures to pulsed 800–915 MHz GSM microwave radiation at a whole-body average SAR of 0.52 W/kg had no effect on the testicular function or structure in rats (Dasdag et al., 2003).

No exposure-related changes were found in exposed or sham-exposed animals with respect to the anogenital distance, preputial separation, testis weight, testicular histology, sperm count, daily sperm production, sperm motility, sperm morphology and reproductive capacity of F1 offspring (Chung et al., 2005). Earlier reports had indicated that prenatal exposure of pregnant rats to EMF resulted in reduction in thyroid and testis weight (Ossenkop et al., 1972).

The main goal of this study was to evaluate the possible effect of whole-body 900- and 1800-MHz EMF exposure on the rat testis

* Corresponding author. Address: Department of Surgery, Faculty of Veterinary Medicine, Ondokuz Mayis University, Kurupelit 55139, Samsun, Turkey.
E-mail address: onisbet@omu.edu.tr (H. Ozlem Nisbet).

0034-5288/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rvsc.2011.10.023

including epididymal sperm concentration, sperm motility, sperm morphology and serum testosterone level in male rats which ranged from 2-day old up to puberty.

2. Materials and methods

The animal study was approved by the Experimental Animal Studies Ethics Committee of Ondokuz Mayis University and was performed. Thirty-three (2 days old) male albino Wistar rats were obtained from the Department of Medical Science Application and Research Center of Ondokuz Mayis University. The rats were randomly divided into three independent groups, 11 of which were exposed to 1800 MHz EMF, 11 to 900 MHz EMF, and 11 of which were sham control rats. The control group of animals was kept under similar conditions except that the field was not applied. The rats were held in a piecage restrainer and then exposed 2 h/day for 90 days, at the same time. The experimental conditions were set up at a temperature of 20 ± 1 °C with a relative humidity of 55% and 12 h of illumination alternated with 12 h of darkness.

2.1. Exposure system and SAR calculation

In this study, groups of rats exposed to 900 and 1800 MHz were kept separately in their experiment boxes. Also, sham exposure group was kept alone isolated electromagnetically. The 900 MHz signal was generated from the same radio frequency source as in Koyu et al., 2005 studies (Everest GSM Simulator (Model: 900CW4, Turkey) and the 1800 MHz signal was obtained from Everest GSM Simulator, another RF source. During exposure, monopole antennas of the exposure systems were arranged and fixed in the closest possible (nearly 2 cm to the head) to the whole body of a rat. In the experiment boxes (with their shielding effectiveness being about 100 dB at 1800 MHz), the radiation of the RF source was checked with a spectrum analyzer, PROMAX, AE-566 with its different probes. The power output of the sources can be controlled by a control knob in their front panel. The power levels antenna output power values of the RF generator exposure system were kept as value of which shows the same effect to the cellular and digital communication handsets commonly used by the general public.

2.2. System integration and exposure plan

An experimental license is required to conduct animal exposure studies at these frequencies, in an unshielded environment, provided the experiment will not cause interference to any licensed wireless communications. Therefore, the experiments were conducted in an RF-shielded room with an estimated attenuation of 100 dB or more to generally comply with existing RF emission limits for devices operating at these frequencies (ICNIRP, 1998). Two different exposure conditions could be provided at the same time. In this setup, different exposure environments were separated and shielded by using specific screens mentioned above. During the experiment, a spectrum analyzer/satellite receiver was used in order to investigate the reflections and background noises in this media. Also the repetition time, frequency, and amplitude of spectrum of the RF energy was investigated, observed, and verified by the satellite level meter, Promax (MC-877C, Barcelona/Spain). All the reflection and exposure measurements were carried out by utilizing Portable RF Survey System, Holaday (HI-4417, MN/USA) with its standard probe as well. The probe is able to pick and get a vector sum on the *X*, *Y* and *Z* axis. It is quite difficult to directly measure the SAR value on an exposed biological tissue. So, the finite-difference time–domain (FDTD) method has been used to compute the SAR values in a simulated environment (Taflove, 1995; Dimbylow, 1997; Gajsek et al., 2002). In this study, the electromagnetic dosimetry solution refers to measured electric field density (V/m) and power density (mW/cm^2) to SARs (digital anatomical models based on the FDTD numerical code).

In this study we marked a design of carousel-like systems used commonly by other researchers to wholebody exposure (Aitken et al., 2005; Koyu et al., 2005; Dasdag et al., 2008). Rats are exposed to electric fields during the growth of rats growing up. The SAR values were calculated by taking into exposed electric field values and account the biological tissue property during the growth of rats growing up. In dosimetric calculation, the dielectric properties and weight of biological material and exposure to electric field is effective. The SAR is expressed in watt per kilogram (W/kg). The SAR given;

$$\mathrm{SAR}(x,y,z) = \sigma(x,y,z)\cdot\frac{[E_{x,y,z}]^2_{\mathrm{rms}}}{\rho} \quad (W/kg)$$

where σ is the electrical conductivity (S m^{-1}) of biological tissue, an induced electric field E in V/m and ρ is the mass density of the tissue (kg/m^3). Dielectric properties vary according to age. For this reason, the study of the dielectric properties made by Peyman and colleagues calculations taking into account (Peyman et al., 2001).

The SAR values for 900 MHz were found to be 3.00, 2.7, 2.2, 1.2 mW/kg for 10, 20, 50, 70 days, adopting σ = 1.2, 1, 0.9, 0.9 S/m, respectively. The SAR values for 1800 MHz were found to be 0.053, 0.046, 0.011, 0.011 mW/kg for 10, 20, 50, 70 days old, adopting σ = 1.7, 1.41, 1.38, 1.39 S/m, respectively. According to the FDTD methodology, the animal was modeled by means of the physical dimension (Sadiku, 2001). Dielectric permittivity and conductivity values of the rat body at certain frequencies were obtained from the scientific reports (Peyman et al., 2001). These SAR calculations were obtained after 500 000 iterations in MATLAB codes.

2.3. Plasma testosterone

Ninety days later, blood sample was collected from the right ventricle of the heart under ether anesthesia. The plasma was separated by centrifugation (3000 rpm for 15 min) and packed in Eppendorf plastic containers (two per sample). The samples were maintained in a freezer at −20 °C. The plasma testosterone concentration was assayed by enzyme-immunoassay (ELISA, enzyme linked immuno sorbent assay). All samples were read in duplicate.

2.4. Cauda epididymal sperm characteristics

After euthanasia by cervical dislocation, (one testicle was used) the caudal epididymidis was quickly removed. The adherent fat, blood vessels, and connective tissues were cut away. The right epididymis was finely minced using anatomical scissors in 1 ml of isotonic saline (0.9%, v/v, NaCl) in a petri dish. It was completely crushed by tweezers for 2 min and then incubated at 36 °C for 5 min to provide the migration of all spermatozoa from the epididymal tissue to the fluid (Kempinas and Lamano-Carvalho 1988; Türk et al., 2008).

The epididymal sperm motility (forward) was evaluated in the Hank's Balanced Salt Solution (HBSS). Under a light microscope (×20 magnification), a random field was chosen and the sperm classified as motile or immotile. Sperm motility was expressed as the percentage of motile sperm per field.

The sperm concentration was determined by a haemocytometer (using Thoma chamber, 0.0025 mm^2 and 0.100 mm depth, Germany) in a 1:100 dilution. The results were presented as sperm cells/ml. To determine the sperm concentration, the diluted samples (1:100) were put into the counting chamber and the number of sperms was counted using a haemocytometer under a light microscope. A total of five squares were counted in both chambers of the haemocytometer and the sum of two counts was divided by

an appropriate factor to get the sperm concentration in millions/ml (Türk et al., 2008).

To determine the percentage of morphologically abnormal spermatozoa, slides stained with Eosine Y (3%, v:v) were prepared. The slides were then viewed under a light microscope at 1000× total magnification. A total of 200 sperm cells were examined on each slide and the head, neck, mid-piece, tail, and total abnormality rates of spermatozoa were expressed as a percentage (Türk et al., 2008; Fernandez et al., 2007).

2.5. Histopathological evaluation of the testis

The other testicle was used for histopathological examination. Seminiferous epithelium and interstitium were evaluated using 5-μm-thick sections stained with haematoxylin and eosin and counter-stained with periodic acid Schiff under light microscopy (Nikon FXA light microscope; Nikon). The diameters of 100 randomly selected essentially round seminiferous tubules from each animal were estimated and classified into one of eight different grades (Veeramachaneni et al., 2006); grade 0, normal intact seminiferous epithelium (Fig. 1); grade 1, seminiferous epithelium with pyknotic cells and desquamation or focal vacuolation (Fig. 3); grade 2, seminiferous epithelium intermediate between grades 1 and 3 (Fig. 2); grade 3, seminiferous epithelium with pre-meiotic germ cells and Sertoli cells; grade 4, Sertoli cells only; grade 5, no seminiferous epithelium, leaving only the basement membrane; grade 6, seminiferous tubule with sperm stasis, sperm granuloma, or mineralization; and grade 7, fibrotic seminiferous tubule. All the histological sections were blindly evaluated by the same investigator (MOK).

2.6. Statistical analysis

The statistical analysis of data was carried out using SPSS statistical package programs. The results of plasma testosterone and caudal epididymal sperm characteristics were expressed as mean ± SEM. Differences between control and exposed groups were analyzed by one-way ANOVA, followed by *Tukey's* and *Dunnett tests*. Histopathological changes analyzed using the Kruskal–Wallis *H*-test and the differences between the groups were determined using the Mann–Whitney *U*-test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Plasma testosterone

The mean plasma total testosterone showed similarity among the two study groups and was significantly higher than the sham control group (Table 1).

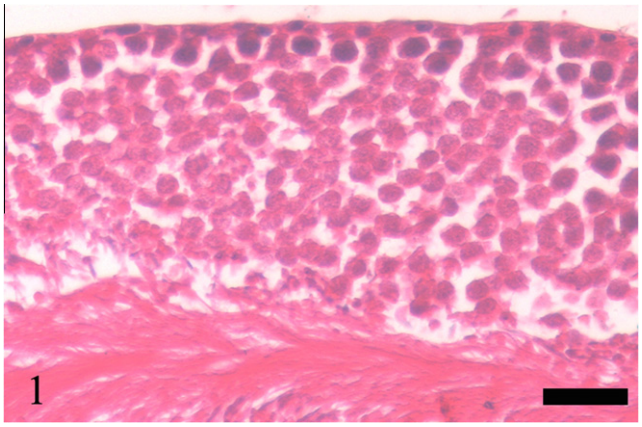

Fig. 1. Case 3 (Control). Intact seminiferous epithelium. Bar: 15 μm.

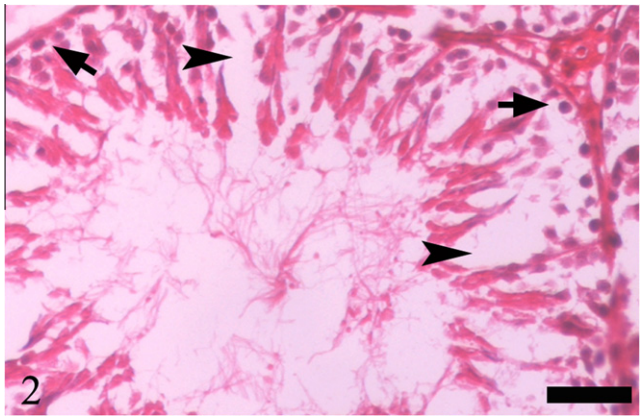

Fig. 2. Case 18 (1800 MHz). Severe vacuolar degeneration of seminiferous epithelium (arrows) and severe necrosis and desquamation (arrow heads). Bar: 30 μm.

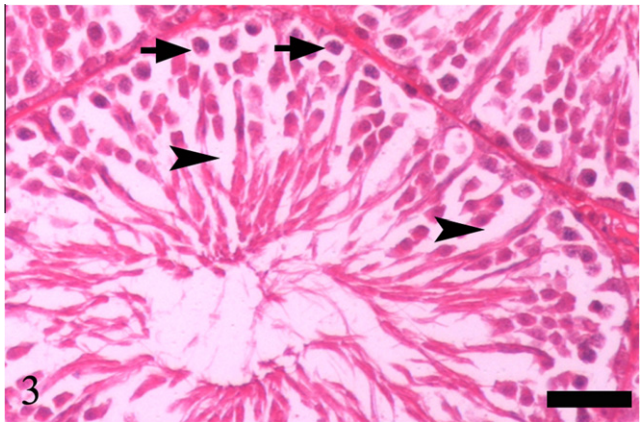

Fig. 3. Case 25 (900 MHz). Vacuolar degeneration of seminiferous epithelium (arrows) and mild desquamation (arrow heads). Bar: 30 μm.

3.2. Cauda epididymal sperm characteristics

The effects of different doses of electromagnetic fields on epididymal sperm concentration, progressive sperm motility (forward), and abnormal sperm rate are presented in Table 2. The percentage of epididymal sperm motility was significantly higher in electromagnetic fields animals ($P < 0.05$) than in sham control animals. There was no statistically significant difference in the sperm concentration, head, and mid-piece abnormalities among the groups. The morphologically normal spermatozoa rates were higher in the 900 MHz group ($P < 0.05$) than in the 1800 MHz group and the sham control group. Tail abnormality and total percentage abnormalities were significantly lower in the 900 MHz group when compared to 1800 MHz group and the sham control group ($P < 0.05$).

Table 1
The level of testosterone concentration of animal groups that were exposed to 1800 and 900 MHz, 2 h daily for 90 days and sham group.

Groups ($n = 11$)	Testosterone levels (ng/ml) mean ± SEM
Control	5.73 ± 0.14
1800 MHz	5.99 ± 0.00*
900 MHz	6.01 ± 0.03*

* Statistical significance, ($P < 0.05$), Tukey test.

Table 2
Effects of electromagnetic waves exposure on cauda epididymal semen parameters in rats treated for 90 days.

Groups ($n = 11$)	Spermatozoa motility (forward) (%)	Sperm concentration ($\times 10^6$/spp./ml)	Normal sperm morphology (%)	Percentage abnormalities			
				Head	Midpiece	Tail	Total percentage abnormalities
Control	50.77 ± 4.15	63.85 ± 6.83	84.00 ± 0.74	4.23 ± 0.30	4.15 ± 0.39	7.92 ± 0.40	16.31 ± 0.75
1800 MHz	64.50 ± 2.17*	80.45 ± 6.63	85.86 ± 0.69	3.73 ± 0.27	3.14 ± 0.22	7.27 ± 0.52	14.14 ± 0.69
900 MHz	64.55 ± 2.47*	75.91 ± 6.54	87.27 ± 0.86*	4.09 ± 0.50	3.77 ± 0.45	4.86 ± 0.46*	12.73 ± 0.86*

* Statistical significance, ($P < 0.05$), the data are expressed as mean ± SEM.

Table 3
Seminiferous epithelium and interstitium of animal groups that were exposed to 1800 and 900 MHz, 2 h daily for 90 days and sham control group.

	Control (mean ± SD)	1800 MHz (mean ± SD)	900 MHz (mean ± SD)
Grades	0.82 ± 0.60	2.27 ± 0.47*	1.45 ± 0.69

* Statistical significance, ($P < 0.05$).

3.3. Histopathological evaluation of the testis

Seminiferous epithelium with pyknotic cells, pre-meiotic germ cells, and sertoli cells and desquamation or focal vacuolation of animals in the 1800 MHz group were significantly higher than both sham control group and 900 MHz group ($P < 0.05$) (Table 3).

4. Discussion

The present study was carried out in rats to determine the potential adverse effects of the pubertal exposure to 900 and 1800 MHz EMF on spermatogenesis and fertility of male rats. The EMF effects on male reproduction are also being investigated.

Nagler and White (1982) reported that in male rats, spermatogenesis begins 15 days after birth and they are fertile 45 days after birth. In our study, the irradiation had been initiated at sexually immature 2 days, even before spermatozoa appeared in the seminiferous epithelium and was sustained to 12 weeks (90 days). There are many doubts about how long is long enough to observe the long term effects of EMF, although, Hecht and Balzer (1997) reported that the long term effects have been given from 200 h up to 20 years in the study of EMF effects.

The major functions of the testes are the production of spermatozoa and the synthesis and release of testosterone. The maturation of germ cells related to testosterone, which released from Leydig cells and the contribution of LH to the maintenance and function of Leydig cells has long been recognized. This androgenic hormone exerts negative feedback effects on gonadotropins at the hypothalamic and pituitary levels (Sarookhani et al., 2011).

Several fertility parameters were affected after exposure to EMF by adult male rats. It is known that the size and function of secondary sex organs are highly dependent on the level of testosterone. Dasdag et al. (2003) found that longer daily exposures to pulsed 800–915 MHz GSM microwave radiation at a whole-body average SAR of 0.52 W/kg had no effect on testicular function or structure in rats.

In the present study, the serum testosterone level was higher due to the exposition to EMF of 900 and 1800 MHz for 2 h per day for 90 days. Forgacs et al. (2006) achieved similar results in their study and their findings indicate that repeated whole-body 1800 MHz GSM-like microwave exposure below the maximum permissible exposure level recommended by ICNIRP is able to increase the serum testosterone level in male mice within the physiological range. Considering their results and the data on the effects of microwaves on the central nervous system, the main targets of action are probably at the higher regulation level of the hypothalamic–pituitary–gonadal axis. The effect of GSM-like microwave exposure on serum testosterone level may be associated with its possible effect on melatonin secretion of the pineal gland. Some recent human data indicate that prolonged use of GSM mobile phones may lead to reduced melatonin production (Burch et al., 2002; Jarupat et al., 2003). It is known that melatonin exerts an antigonadotrophic effect mainly at the level of the hypothalamus and pituitary (Jackson et al., 1984; Bittman et al., 1985; Vanecek, 1998) and directly decreases the testosterone secretion in Leydig cells too (Ozguner et al., 2002; Kim et al., 2009).

It was previously reported that evaluation of testicular sperm head counts seems to be a good indicator of spermatogenic damages and the number of testicular sperm heads corresponds to the number of elongate spermatids in the testis (Meistrich, 1989; Kim et al., 1999; Fejes et al., 2005). Although various sperm abnormalities such as small head, amorphous head, two heads/tails, excessive hook, blunt hook, folded tail, short tail and no tail were observed in the both exposed and sham-exposed groups, there was no obvious difference in the incidence of head and midpiece abnormalities between the groups. Only tail abnormality was lower in the 900 MHz group.

The sperm concentration in treated groups presented an increase in relation to the control. Although this difference was not statistically significant, this change and significant increase in spermatozoa motility are noticed in this project may be indicative of some closeness to precocious puberty in the animals of these groups. Additionally, clinical observations of the study showed that 1800 and 900 MHz group of rats grew faster and lose more weight (data not shown). True precocious puberty is the result of premature initiation of the function of the hypothalamic–pituitary–gonadal axis. As a consequences, the gonads function at an inappropriately early age manifesting as early spermatogenesis and other secondary sex characters (Zareen, 2009).

In the study, the abnormal cellular morphology observed in the experimental groups could be due to the thermal effects to the EMFs. Zareen (2009) achieved similar results in his study and their findings indicate that histological comparison of testis of the treated group and control group animals showed in significant increase, in the number of tubules with sperms in the lumen, presence of vacuolation and giant cells in germinal epithelium and abnormal cells in the lumen of seminiferous tubules of the treated group.

In our study, an increase in epididymal sperm motility and normal sperm morphology were observed as well as an increase in testosterone concentration in EMF-applied groups. It is thought that this condition was due to a decline in melatonin concentration and an increase in testosterone concentration. Based on these findings it was concluded that EMF-applied groups reached puberty earlier. Particularly, the group in which 900 MHz EMA was applied revealed a distinct alteration in the caudal epididymal sperm characteristics. Similarly, Ozguner et al. (2002) observed that EMF stimulation resulted in leydig cell proliferation, increased testosterone level, and testis weight.

5. Conclusion

The present study indicated that exposure to electromagnetic wave caused an increase in testosterone level, epididymal sperm motility, and normal sperm morphology of male rats. Additionally, the sperm morphology (sperm head and sperm tail) and motility were more sensitive to EMF exposure than the sperm concentration. The results of the present study demonstrated that 1800 and 900 MHz EMF could be considered to be a cause of precocious puberty in growing rats.

Considering the results of the study, further studies by including before- and after-puberty periods at even longer time intervals are necessary to clarify the mechanisms of EMF.

Acknowledgements

The authors would like to thank Dr. Oguzhan Yavuz, Department of Pharmacology, University of Ondokuz Mayis, Faculty of Veterinary Medicine, Samsun, Turkey, for his valuable help in statistical analysis and Assist. Prof. Dr. Mesut Kahraman from Suleyman Demirel University, Electronic and Communication Engineering Department because of supplying us the software used in the calculation of SAR value. This study was supported by Scientific Research and Development Support Program of University of Ondokuz Mayis.

References

Aitken, R.J., Bennetts, L.E., Sawyer, D., Wiklendt, A.M., King, B.V., 2005. Impact of radio frequency electromagnetic radiation on DNA integrity in the male germline. International Journal of Andrology 28, 171–179.

Al-Akhras, M.A., Elbetieha, A., Hasan, M.K., Al-Omari, I., Darmani, H., Albiss, B., 2001. Effects of extremely low frequency magnetic field on fertility of adult male and female rats. Bioelectromagnetics 22, 340–344.

Atmaca, S., Akdağ, Z., Daşdağ, Çelik.S., 1996. Effect of microwave on survival of some bacterial strains. Acta Microbiologica et Immunologica Hungarica 43, 371–378.

Bittman, E.L., Kaynard, A.H., Olster, D.H., Robinson, J.E., Yellow, S.M., Karsch, F.J., 1985. Pineal melatonin mediates photoperiodic control of pulsatile luteinizing hormone secretion in the ewe. Neuroendocrinology 40, 409–418.

Burch, J.B., Reif, J.S., Noonan, C.W., Ichinose, T., Bachand, A.M., Koleber, T.L., Yost, M.G., 2002. Melatonin metabolite secretion among cellular telephone users. International Journal of Radiation Biology 78, 1029–1036.

Chung, M.K., Lee, S.J., Kim, Y.B., Park, S.C., Shin, D.H., Kim, S.H., Kim, J.C., 2005. Evaluation of spermatogenesis and fertility in F1 male rats after in utero and neonatal exposure to extremely low frequency electromagnetic fields. Asian Journal of Andrology 7 (2), 189–194.

Dasdag, S., Ketani, M.A., Akdag, Z., Ersay, A.R., Sari, I., Demirtas, O.C., Celik, M.S., 1999. Whole-body microwave exposure emitted by cellular phones and testicular function of rats. Urological Research 27, 219–223.

Dasdag, S., Akdag, M.Z., Aksen, F., Yilmaz, F., Bashan, M., Dasdag, M., Celik, S., 2003. Whole body exposure of rats to microwaves emitted from a cell phone does not affect the testes. Bioelectromagnetics 24, 182–188.

Dasdag, S., Akdag, M.Z., Ulukaya, E., Uzunlar, A.K., Yegin, D., 2008. Mobile phone exposure does not induce apoptosis on spermatogenesis in rats. Archives of Medical Research 39, 40–44.

De Vita, R., Cavallo, D., Raganella, L., Eleuteri, P., Grollino, M.G., Calugi, A., 1995. Effects of 50 Hz magnetic fields on mouse spermatogenesis monitored by flow cytometric analysis. Bioelectromagnetics 16, 330–334.

Dimbylow, P.J., 1997. FDTD calculations of the whole-body averaged SAR in an anatomically realistic voxel model of the human body from 1 to 1 GHz. Physics in Medicine and Biology 42, 479–490.

Fejes, I., Zavaczki, Z., Szollosi, J., Koloszar, S., Daru, J., Kovacs, L., Pal, A., 2005. Is there a relationship between cell phone use and semen quality? Archives of Andrology 51 (5), 385–393.

Fernandez, C.D.B., Porto, E.M., Arena, A.C., Kempinas, W.G., 2007. Effects of altered epididymal sperm transit time on sperm quality. International Journal of Andrology 31, 427–437.

Forgacs, Z., Somosy, Z., Kubinyi, G., Bakos, J., Hudak, A., Surjan, A., Thuroczy, G., 2006. Effect of whole-body 1800 MHz GSM-like microwave exposure on testicular steroidogenesis and histology in mice. Reproductive Toxicology 22, 111–117.

Gajsek, P., Walters, T.J., Hurt, W.D., Ziriax, J.M., Nelson, D.A., Mason, P.A., 2002. Empirical validation of SAR values predicted by FDTD modeling. Bioelectromagnetics 23, 37–48.

Hecht K, Balzer HU., 1997. Biological effects of electromagnetic fields on humans in the frequency range of 0 to 3 GHz. Berlin, I.S.F. 1–4.

Heredia-Rojas, J.A., Caballero-Hermandez, D.E., Rodriguez-de la Fuente, A.O., Ramos-Alfano, G., Rodriguez-Flores, L.E., 2004. Lack of alterations on meiotic chromosomes and morphological characteristics of male germ cells in mice exposed to a 60 Hz and 2.0 mT magnetic field. Bioelectromagnetics 25, 63–68.

ICNIRP., 1998. Guidelines for Limiting Exposure to Time-Varying Electric, Magnetic, and Electromagnetic Fields (up to 300 GHz). Health Physics 74 (4), 494–522.

Jackson, F.L., Heindel, J.J., Preslock, J.P., Berkowitz, A.S., 1984. Alterations in hypothalamic content of luteinizing hormone-releasing hormone associated with pineal mediated testicular regression in the golden hamster. Biology of Reproduction 31, 436–445.

Jarupat, S., Kawabata, A., Tokura, H., Borkiewicz, A., 2003. Effects of the 1900 MHz electromagnetic field emitted from cellular phone on nocturnal melatonin secretion. Journal of Physiology and Anthropology 22, 61–63.

Kempinas, W.G., Lamano-Carvalho, T.L., 1988. A method for estimating the concentration of spermatozoa in the rat cauda epididymidis. Laboratory Animals 22, 154–156.

Kim, J.C., Kim, K.H., Chung, M.K., 1999. Testicular cytotoxicity of DA-125, a new anthracycline anticancer agent, in rats. Reproductive Toxicology 13, 391–397.

KimYW, Kim.H.S., Lee, J.S., KimYJ, Lee., SK, Seo.J.N., Jung, K.C., Kim, N., Gimm, Y.M., 2009. Effects of 60 Hz 14 mT magnetic field on the apoptosis of testicular germ cell in mice. Bioelectromagnetics 30, 66–72.

Kolosova, L.I., Akoev, G.N., Avelev, V.D., Riabchikova, O.V., Babu, K.S., 1996. Effect of low-intensity millimeter wave electromagnetic radiation on regeneration of sciatic nerve in rats. Bioelectromagnetics 17, 44–47.

Koyu, A., Cesur, G., Özgüner, F., Akdogan, M., Mollaoglu, H., Ozen, Ş., 2005. Effects of 900 MHz electromagnetic field on TSH and thyroid hormones in rats. Toxicology Letters 157, 257–262.

Lai, H., Singh, N.P., 1995. Acute low intensity microwave exposure increases DNA single-strand breaks in rat brain cells. Bioelectromagnetics 16, 207–210.

Lai, H., Singh, N.P., 1997. Melatonin and a spin-trap compound block radiofrequency electromagnetic radiation-induced DNA strand breaks in rat brain cells. Bioelectromagnetics 18, 446–454.

Lee, J.S., Ahn, S.S., Jung, K.C., Kim, Y.W., Lee, S.K., 2004. Effects of 60 Hz electromagnetic field exposure on testicular germ cell apoptosis in mice. Asian Journal of Anrology 6, 29–34.

Meistrich, M.L., 1989. Evaluation of reproductive toxicity by testicular sperm head counts. Journal of American College Toxicology 8, 551–566.

Nagler, H.M., White, R.D., 1982. The effect of testicular torsion on the contralateral testis. Journal of Urology 128, 1343–1348.

Ossenkop, K.P., Koltek, W.T., Persinger, M.A., 1972. Prenatal exposure to an extremely low frequency, low intensity rotating magnetic field and increase in thyroid and testicle weight in rats. Developmental Pshycobiology 5, 275–285.

Ozguner, I.F., Dindar, H., Yagmurlu, A., Savas, C., Gokcora, I.H., Yucesan, S., 2002. The effect of electromagnetic field on undescended testis after orchiopexy. International Urology and Nephrology 33, 87–93.

Peyman, A., Rezazadeh, A., Gabriel, C., 2001. Changes in the dielectric properties of rat tissue as a function of age at microwave frequencies. Physics in Medicine and Biology 46, 1617–1629.

Roberts, M.R., Michaelson, J.S.M., Lu, S.T., 1986. The biological effects of radiofrequency radiation: a critical rewiev and recommendations. International Journal of Radiation Biology 50, 379–420.

Rojavin, M.A., Ziskin, M.C., 1995. Effect of milimeter waves on survival of UVC-exposed *Escherichia coli*. Bioelectromagnetics 16, 188–196.

Sadiku, M., 2001. Elements of Electromagnetics. Oxford University Press, New York.

Sarookhani, M.R., Rezaei, M.A., Safari, A., Zaroushani, V., Ziaeiha, M., 2011. The influence of 950 MHz magnetic field (mobile phone radiation) on sex organ and adrenal functions of male rabbits. African Journal of Biochemistry Research 5 (2), 65–68.

Taflove, A., 1995. Computational Electrodynamics: The Finite-Difference Time–Domain Method. Artech House, Norwood, MA.

Türk, G., Sönmez, M., Aydin, M., Yüce, A., Gür, S., Yüksel, M., Aksu, E.H., Aksoy, H., 2008. Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. Clinical Nutrition 27, 289–296.

Vanecek, J., 1998. Melatonin inhibits release of luteinizing hormone (LH) via decrease of [Ca2+]*i*, and cyclic AMP. Physiological Research 47, 329–335.

Veeramachaneni, D.N., Palmer, J.S., Amann, R.P., Kane, C.M., Higuchi, T.T., Pau, K.Y., 2006. Disruption of sexual function, FSH secretion, and spermiogenesis in rabbits following developmental exposure to vinclozolin, a fungicide. Reproduction 131, 805–808.

World Health Organization (WHO), 1987. Environmental Health Criteria 69: Magnetic fields. Geneva.

Zareen, N., 2009. Testicular morphology: effects of mobile phone induced electromagnetic fields on mice testes. Professional Medical Journal 16 (2), 289–292.