Biological and morphological effects on the reproductive organ of rats after exposure to electromagnetic field

***Meltem Ozguner**, MD, **Ahmet Koyu**, MD, **Gokhan Cesur**, PhD, **Mehmet Ural**, MD, **Fehmi Ozguner**, MD, **Alpaslan Gokcimen**, MD, **Namık Delibas**, MD.*

ABSTRACT

Objectives: The biological effect of electromagnetic field (EMF) emitted from mobile phones is a current debate and still a controversial issue. Therefore, little is known on the possible adverse effects on reproduction as mobile phone bio-effects are only a very recent concern. The aim of this experimental study was to determine the biological and morphological effects of 900 MHz radiofrequency (RF) EMF on rat testes.

Methods: The study was performed in the Physiology and Histology Research Laboratories of Süleyman Demirel University, Faculty of Medicine, Isparta, Turkey in May 2004. Twenty adult male Sprague-Dawley rats weighing 270 - 320 gm were randomized into 2 groups of 10 animals: Group I (control group) was not exposed to EMF and Group II (EMF group) was exposed to 30 minutes per day, 5 days a week for 4 weeks to 900 MHz EMF. Testes tissues were submitted for histologic and morphologic examination. Testicular biopsy score count and the percentage of interstitial tissue to the entire testicular tissue were registered. Serum testosterone, plasma luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels were assayed biochemically.

Results: The weight of testes, testicular biopsy score count and the percentage of interstitial tissue to the entire testicular tissue were not significantly different in EMF group compared to the control group. However, the diameter of the seminiferous tubules and the mean height of the germinal epithelium were significantly decreased in EMF group ($p<0.05$). There was a significant decrease in serum total testosterone level in EMF group ($p<0.05$). Therefore, there was an insignificant decrease in plasma LH and FSH levels in EMF group compared to the control group ($p>0.05$).

Conclusion: The biological and morphological effects resulting from 900 MHz RF EMF exposure lends no support to suggestions of adverse effect on spermatogenesis, and on germinal epithelium. Therefore, testicular morphologic alterations may possibly be due to hormonal changes.

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Since electromagnetic fields (EMFs) are present everywhere all over the world and are produced by either natural sources or human made sources, there is a growing interest in the potential biological effects on both human and animal health. During the 20th century, environmental exposure to human made sources of EMFs has been steadily increasing as growing electricity demand, ever-advancing technologies and changes in social behavior have created many artificial sources including power lines, microwave ovens, computers and television, security devices, radars and most recently mobile phones and their base stations.

The dominant access technique in Europe is the so-called Time Division Multiple Access (TDMA)

From the Department of Histology and Embryology (Ozguner M, Ural, Gokcimen), Department of Physiology (Koyu, Cesur, Ozguner F) and the Department of Biochemistry and Clinical Biochemistry (Delibas), Faculty of Medicine, Suleyman Demirel, University, Isparta, *Turkey*.

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Address correspondence and reprint request to: Dr. Meltem Ozguner, Kurtulus Mah. 137. Cad. Eralp Apt. 7/9, Isparta 32040, *Turkey*. Tel. +90 (246) 2113244. Fax. +90 (246) 2371758. E-mail: mozguner@hotmail.com

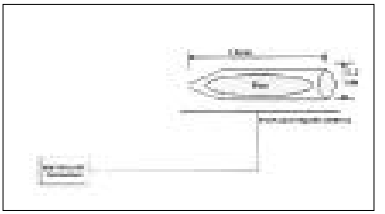

Figure 1 - The 900 MHz radiofrequency exposure device.

Figure 3 - Representative light micrograph of testicular interstitium with interstitial edema after 900 MHz exposure (original magnification x100, hematoxylin and eosin stained)

Figure 2 - Representative light micrograph of rat testes after 900 MHz elecromagnetic field exposure. Though seminiferous epithelium disorganized, mature germ cells present in the lumen of seminiferous tubules representing complete spermatogenesis (original magnification x200, hematoxylin and eosin stained).

Figure 4 - Representative light micrograph of rat testes tissue in control group (original magnification x100, hematoxylin and eosin stained)

technique, which is used in Global System for Mobile communication (GSM), Digital European Cordless Telecommunications (DECT), Digital personal Communication System 1800 (DCS) and in Trans European Trunked Radio (TETRA) systems.[1] The carrier frequency bands allocated for these services are set mainly in the spectrum regions of 800 - 900 MHz and 1.8 - 2.2 GHz.[2] Although many animal studies have attempted to determine the effects of exposure to EMFs on reproduction, the results were rather contradictory. Many studies concluded that exposure to EMFs can have adverse effects on reproduction and fetal development[3-11] and a relationship between chronic or long term exposure to EMFs and fetal loss has been suggested.[12-14] Other studies have reported no adverse effects on reproduction, fertility and histology of testes in rats, mice and human exposed to EMFs.[15-21] Some studies related to EMFs emitted from mobile phones have reported biological adverse effects.[22-24] Considerable attention has recently been given to investigate whether EMFs emitted by mobile phones could cause adverse effects on male reproductive system and some studies have reported that there is no evidence suggesting an adverse effect of mobile phone exposure on testicular function or structure.[25] It is clear, that the biological effect of mobile phone exposure may be variable depending on exposure periods, conditions, species and tissues. In this study, we investigated whether 900 MHz continuous wave, average power density 1.04 Mw/cm^2 EMF emanating from a dipole antenna as used in GSM have effects on testes morphology and serum total testosterone, plasma luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels.

Methods. ***Animals.*** Twenty adult male Sprague-Dawley rats (Experimental Research Unit, Faculty of Medicine, Selcuk University, Turkey), with initial weights of 270 - 320 gm (5 months of age), were caged individually and fed with standard pellet food. They were maintained under controlled temperature of 21 ± 1^{o}C in 12 hours light and 12 hours darkness schedule. Food and tap water were freely available ad libitum throughout the experiment.

Table 1 - Testicular biopsy score count (Johnsen score).

Score	Histologic findings
10	Complete spermatogenesis, numerous spermatozoa, germinal epithelium of regular height, tubular lumen of normal diameter
9	Numerous spermatozoa, germinal epithelium disorganized with sequestration of germinal cells, tubular lumen obturated
8	Less than 5 ± 10 spermatozoa per tubular cross-section
7	No spermatozoa, numerous spermatids, spermatocytes and spermatogonia
6	No spermatozoa, 5 ± 20 spermatids, numerous spermatocytes and spermatogonia per cross-section
5	No spermatozoa and spermatids, numerous spermatocytes and spermatogonia
4	No spermatozoa and spermatids, less than 5 spermatocytes, but numerous spermatogonia per cross-section
3	Only spermatogonia
2	No germinal cells, only Sertoli cells (Sertoli-cell-only syndrome)
1	No cells at all within the tubules

Experimental setup and electromagnetic field exposure. A 900 MHz continuous wave electromagnetic energy generator (2 watts peak power, average power density 1 ± 04 mW/cm²) was used in the study. The power density measurements were made using Holaday Industry, Incorporated EMF meter was produced at the electromagnetic compatibility (EMC) laboratory of Sakarya University (Adapazarı, Turkey).

The exposure system consisted of a plastic tube cage (length: 12 cm, diameter: 5.5 cm) and a dipole antenna (**Figure 1**). The whole body of the rats was positioned in close contact above the dipole antenna, and the tube was ventilated from head to tail, to decrease the stress of the rat while in the tube.

Animals were randomly grouped as follows: Control group (n=10) and EMF group (n=10). Rats exposed 30 minutes a day, for 5 days a week for 4 weeks to a 900 MHz EMF were compared to control rats. Control animals were fed under the same environmental conditions as the exposure group.

Histopathologic examination. Testes were removed for histopathological examination. The removed testes were bisected and fixed in 10% formaldehyde solution. The tissue samples were embedded in paraffin and 5 µm cross-sections were stained with hematoxylin-eosin. Histologic sections were assessed and photographed by Olympus BX50 (Japan) photomicroscope. The following parameters were evaluated to assess the extent of testicular changes: 1. The weights of paired testes were recorded. 2.The mean diameter of round seminiferous tubules randomly selected in each cross-section and the mean height of the germinal epithelium were measured in 30 cross-sections per animal by using an ocular micrometer. 3. Spermatogenesis was determined by the semiquantitative 'testicular biopsy score count'[26] (Johnsen score, **Table 1**) in 100 cross-sections in each animal at the same magnification and was summed up as mean Johnsen testicular biopsy score (MJS). 4. The percentage of interstitial tissue and of Leydig cells to the entire testicular tissue was registered by the stereologic technique of Weibel and Gomez, [27] which is based on a network of 100 regularly distributed points focused onto the microscopic screen. General histologic findings of the testes were also evaluated for each group.

Hormone analysis. To analyze testosterone hormone, blood samples were collected into heparinized tubes from all animals on decapitation, and separated by centrifugation at 3000 rpm for 10 minutes and serum was stored at −70°C until use. Total testosterone was analyzed using double-antibody radioimmunoassay method by Coat-A-Count Total Testosterone Kit (Diagnostic Products Corporation). Plasma LH and FSH levels were measured by homologous specific double antibody radioimmunoassay (Chiron Diagnostics, Auto Chemiluminescence System, Indianapolis, United Stated of America).

Statistical analysis. Statistical Package for Social Sciences version 9 for Windows used for statistical analysis. The data are presented as means ± standard error of mean. All results in the study were analyzed by Mann-Whitney U test, with $p<0.05$ as the criterion for significance for all statistical comparisons.

Results. At the end of the study the mean weights of testes were not significantly different in EMF group compared to control group. The average weight of testes for the control group and EMF group are presented in **Table 2**.

The diameter of the seminiferous tubules was significantly reduced in EMF group compared to control group (**Table 2**). In EMF group, the mean height of the germinal epithelium was 50 µm, in control group it was 68.50 µm ($p<0.05$) (**Table 2**). However, estimation of spermatogenesis by application of the Johnsen testicular biopsy score revealed no statistically significant difference between EMF and control groups (**Table 2**). The percentage of interstitial tissue in the entire testicular parenchyma has been increased slightly but it was not statistically significant in EMF group compared to control group ($p>0.05$) (**Table 2**). In EMF group, conglomerates of germ cells were seen

Table 2 - Comparison of morphological parameters between control and electromagnetic field group.

Groups	Testes weight (g)	STD (µm)	GEH (µm)	MJS	Percentage of interstitial tissue
Control	1739.8 ± 61.2	261.6 ± 9.8	68.5 ± 2.6	9.6 ± 0.2	38 ± 2.2
EMF	1715.0 ± 73.7	233.7 ± 12.4*	59 ± 2.7	9.5 ± 0.2	40 ± 1.8

STD - Seminiferous tubules diameter, GEH - Germinal epithelium height, MJS - mean Johnsen testicular biopsy score, EMF - Electromagnetic field. *Statistically significant difference between 2 groups (p<0.05).

Table 3 - Comparison of total testosterone, luteinizing hormone and follicle stimulating hormone levels between control and electromagnetic field group.

Groups	Total testosterone (ng/dl)	Luteinizing hormone (ng/ml)	Follicle stimulating hormone (ng/ml)
Control	228 ± 23.2	0.7 ± 0.12	5.4 ± 0.53
EMF	152.5 ± 9.02*	0.4 ± 0.06	4.8 ± 0.29

EMF - Electromagetic field. *Statistically significant difference between 2 groups (p<0.05).

in the lumen of seminiferous tubules. There was no significant degeneration of germinal epithelium (**Figure 2**). Additionally, the interstitial cellularity was normal, and there were no evidence of inflammation or interstitial fibrosis in EMF group (**Figure 3**). However, there were interstitial edema in EMF group compared to control group (**Figures 3 & 4**).

In contrast to interstitial tissue histology, serum testosterone was significantly reduced in EMF group compared to control group (p<0.05) (**Table 3**). Therefore, there were no statistically significant differences in plasma LH and FSH levels in EMF group when compared with the control group (p>0.05) (**Table 3**).

Discussion. Electro magnetic field exposure at 900 MHz radiofrequency was selected for this experiment as this was the carrier frequency used in most of the GSM systems. Possible health effects of 900 MHz EMF were reported mostly considering neuroendocrine system.[28-30] The number of studies into the possible effects of 900 MHz EMF exposure on testes is, however, very few and outcomes are very contradictory.[18,25,31] In a previous study, mobile phone exposure of rats did not result in alteration of testes histology.[25] Just contrary, another study of the same author, exposure of rats to microwaves emitted by the phones with a carrier frequency 890 - 915 MHz, 217 Hz modulation frequency, 2 watt maximal peak power for 2 hours per day for a duration of one month accompanied with histologic alterations.[31] The observations of the present study indicated weight of testes and spermatogenesis was not affected in 900 MHz EMF exposure. Though, the mean diameter of seminiferous tubules and the mean height of germinal epithelium were reduced. Tubular diameter and epithelial height are larger if the more mature the germinal cells appear.[32] Saunders[33] has reported 1.7 GHz EMF resulted significant degeneration of germinal epithelium in mice. Ozguner et al[34] concluded that 8.64 mili Tesla EMF stimulation resulted in Leydig cell proliferation, increase in testosterone level, testis weight, but decrease in germ cell population. However, in the present experimental set-up, intermittent 900 MHz exposure for one month did not cause any degenerative changes on germinal epithelium. The different testicular tissue effects resulting from EMF exposure were possibly depending upon the power density of source and frequency of radiation.

According to the literature,[35] the percentage of interstitial tissue in the entire testicular parenchyma ranges from 31 - 41% and can absolutely increase by active proliferation of fibers and cellular elements during inflammation, through hormonal stimulation from the hypophysis, or by tumor growth. On the other hand, it has been demonstrated that EMF could activate fibroblasts and result the acceleration of collagen synthesis and wound healing.[36] In our study, a relative increase in the percentage of interstitial tissue was due to interstitial edema, which often evolved after local vasodilatation. Moreover, Paredi et al[37] reported that 900 MHz microwaves may cause vasodilation.

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. In this study, biochemical analysis revealed that serum total testosterone was decreased in EMF group compared to control group. Serum total testosterone level may decrease either due to lack of hypophyseal stimulation or to disturbed testicular functions preventing differentiation of Leydig cells from mesenchymal cells. However, Seze et al [38] reported that radiocellular telephones do not disturb the secretion of anterior pituitary hormones in humans, although the authors did not measure testosterone hormone level. Our results confirm that serum testosterone level significantly decreased although plasma LH and FSH levels slightly decreased. Though, low testosterone level was expected to lead higher levels of FSH and LH by feed-back mechanisms. In our study, low testosterone levels but normal FSH and LH levels may be explained by minimal effect of EMF on hypophysis. This may lead to inhibition of excessive FSH and LH release. Hormonal imbalance and dysregulation at the hypothalamic or hypophyseal level might led to development of secondary hypogonadism, however, it was not consistent with the findings on interstitial cells. As spermatogenesis did not mainly effected in this experimental model, direct adverse effect on testes was not supposed. Though, this assessment also needs confirmation. Dasdag et al[25] found no evidence suggesting an adverse effect of cell phone exposure on testes histology, p53 immune reactivity, malondialdehyde concentrations, sperm counts, sperm morphology, and rectal temperature of rats after cellular phone activation for 20 minutes per day (7 days a week) for one month. The duration of exposure and frequency of radiation in our study were similar with this previous study. Additionally, we have evaluated LH, FSH and testosterone levels, which directly affect testicular function. Some experiments have shown that acute and chronic low EMF exposure of conscious mice and rats very often do not alter the testicular function or fertility.[39] Although at high exposures, temperature-mediated effects cannot be excluded, it is commonly accepted that EMFs emitted by mobile phones are at non-thermal power density level.[31]

The World Health Organization established the international EMF Project in 1996 and a fact sheet updating conclusions and recommendations regarding health effects from mobile phone use and exposure to base stations was published in June 2000.[40] It briefly states that none of the recent reviews have concluded that exposure to the radiofrequency (RF) fields from mobile phones or their base stations causes any adverse health consequence. However, there are gaps in knowledge that have been identified for future research to better assess health risks.

Laboratory studies on animals play an essential role in evaluating the integrated response of various systems of the body, particularly the nervous, endocrine and reproductive systems. Therefore, as the number of animal studies increases, this will provide the opportunity to contribute health risk assessments of EMF exposure.

Biological effects are measurable responses to a stimulus or to a change in the environment and an adverse effect causes detectable impairment of the exposed individual. A biological effect, on the other hand, may or may not result in an adverse health effect. In conclusion, whole body exposure of rats to 900 MHz RF EMF 30 minutes per day for 5 days a week for 4 weeks causes biological effects on testes tissue but it does not cause an adverse effect on spermatogenesis. Therefore, our results support the view that this biological effect may be due to hormonal changes rather than direct morphological alterations. Extrapolation of these results to man would suggest that long term exposure to 900 MHz EMF emitted from mobile phones may have biological effect on testes. However, there was insufficient data to comment on adverse effects on fertility.

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