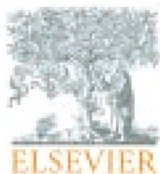

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Low frequency electromagnetic fields long–term exposure effects on testicular histology, sperm quality and testosterone levels of male rats

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ABSTRACT

Objective: To evaluate the effects of long-term exposure to low frequency EMF on the testicular function and structure. **Methods:** Fourteen adult male rats were randomly and equally divided into sham and experimental groups. Experimental group was exposed to 1 mT, 50 Hz EMF, continuously for 85 days in a solenoid. Sham group was kept under conditions same as experimental group, without EMF. At the end of the exposure period, weight and size of testes, sperm evaluation (sperm counts, motility and viability), histological testicular sections and serum total testosterone were determined. **Results:** Long-term exposure to low frequency EMF significantly decreased the diameter of the seminiferous tubules and increased number of seminiferous tubules per unit area of testes. In addition, low frequency EMF significantly reduced sperm motility and testosterone levels. However, it had no effect on the weight and size of testes, sperm concentration, and viability. **Conclusion:** Prolonged exposure to 50 Hz EMF has an adverse effect on male fertility.

1. Introduction

Developments in technology and industry have simplified human life. However, exposure to electromagnetic fields (EMFs) by using electrical machines, tools, industrial instruments, power lines, and communications devices has occurred as a result of these technological developments and is causing a threat to normal lives. The testis, organ of the male reproductive system, where sperm and testosterone are produced, is very sensitive to a variety of factors such as hyperthermia, inflammation, radiation and exposure to agents that lead to apoptosis of germ cells [1].

Some studies have reported that EMF can have adverse effects on reproduction and fertilizing potential of spermatozoa, while, a number of studies showed that exposure to EMF did not induce any adverse effects on the reproductive capacity. Moreover, the reports about effects of EMF on testosterone level vary that are associated with magnetic fields densities and the time of exposure.

Use of cell phones by men or exposing it to the rat reduced the semen parameters by decreasing the sperm count, motility, viability, and normal morphology[2-5]. However, Gutschi *et al.* [6] reported the same results including increase in testosterone concentration, but they did not observe change in sperm count. In contrast, exposure to mobile phone radiation (900 MHz), 30 minutes per day, 5 days a week for 4 weeks leads to decrease in serum testosterone levels [7]. However, exposure to EMF did not induce any adverse effects on sperm quantity, quality, and morphology, but decreased testosterone levels in rats [8-10]. In contrast, exposure to EMF (1800 and 900 MHz) 2 h continuously per day for 90 days [11] and exposure to 1800 MHz GSM-like [12] caused an increase in testosterone level. Otherwise, radiofrequency EMF, 1 h/day for 2 weeks did not induce any adverse effects on the sperm quality [8, 13]. In addition, exposure to circularly polarized, 50 Hz magnetic fields continuously for 6 weeks in rats [14], exposure to 50 Hz static magnetic fields, 40 minutes daily for 17 days [15] and exposure to 50 Hz, 5 mT magnetic

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field for periods of 1,2 and 4 weeks [16] represented that have no effects on testosterone level of male rats significantly.

On the other hand, jammer radiofrequency radiation decreased sperm motility in men [17]. In addition, exposed to an internet-connected laptop by Wi-Fi for 4 h significantly reduced sperm motility[18]. Moreover, Wistar rats exposed to Laptop Computers' EMF (1.15 μT) for 7 h/day for 1 week reduced sperm count and motility[19].

Since, humans in modern society are exposed to low frequency of EMFs, generated by power lines and household electric appliances, during boarding, the aims of the present study were to evaluate the effect of 50 Hz EMF for 85 days, 24 h/day on testosterone level, semen evaluation indices and stereological parameters of testis in male rats.

2. Materials and methods

2.1. Animals

The experimental protocol was performed based on the Animal Care and Use Protocol, Shiraz University, Shiraz, Iran. Fourteen male Sprague-Dawley rats at an average weight of 160-180 g were used. The rats were maintained under the stable condition at room temperature (22-25 ℃, 12-hour light/dark, photo schedule); standard laboratory animal feed and water were provided to animal ad libitum. The rats were adapted to laboratory condition since 7-days before beginning of the study.

2.2. Animal treatment

NiRats were randomly divided into two groups, sham and experimental groups. Experimental group was exposed to 1 mT, 50 Hz low frequency EMF, for 85 days 24 h/day in a solenoid. Sham group was kept under conditions similar to experimental group, without EMF.

2.3. Electromagnetic fields inducing system

Continuous 50 Hz EMF were produced by magnetic coils. The solenoid was attached with 600 turns of 1 mm copper wire on a wooden framework. The solenoid was connected to an autotransformer, with a voltage percent scale, which was connected to 220 V power. Calibration of the system was accomplished by a digital electromagnetic field tester (EMF 827, Lutron). Cages with animals were placed symmetrically on both sides of the coils.

2.4. Testosterone hormone measurement

At the end of the exposure period, the rats were anaesthetized by ether. Blood samples were collected via cardiac puncture, stored in tubes without anticoagulants and allowed to clot. The clotted blood samples were centrifuged at 2 000 rpm for 15 minutes to obtain the serum. Serum was stored at -20 ℃ until analysis. Serum testosterone level was measured by radioimmunoassay (RIA) technique (DIA source ImmunoAssays, S.A.). The sensitivity of hormone detected per assay tube was 0.05 ng/mL.

2.5. Epididymis sperm preparation and sperm quality evaluation

The procedure used for collecting and analyzing semen samples was based on Seed *et al.*[20] To obtain semen samples, the epididymis was immediately separated. Approximately a 1-cm portion of the distal end of the vas deferens was excised and placed in a Petri dish containing 5 mL pre-warmed Hank's Balanced Salt Solution buffer (HBSS), transferred to an incubator at 37 ℃ and gently swirled the Petri dish for 10 min to facilitate the spontaneous release of sperm from the vas deferens. To estimate of the percentage of motile sperm within samples, semen samples slides were evaluated with light microscopy in randomly 10 selected fields with a 40 objective. The mean sperm counts were determined via microscopic examination. Briefly, the semen samples were diluted, and transferred to the Improved Neubauer chamber. Then, the number of sperm counted in large squares within central counting area of a chamber was calculated. Furthermore, sperm viability was evaluated by use of eosin-nigrosin staining. Briefly, a fraction of each sperm suspensions were mixed with an equal volume of 0.5% eosin-nigrosin solution and smears on a glass microscope slide then they were evaluated with light microscopy in randomly 10 selected fields microscope for the percentage of vital (unstained) and dead (stained) spermatozoa.

2.6. Stereological analysis

The right testes of both groups were dissected out, weighed using a digital weighing scale (Acculab ALC210.4), whereas the length and diameter of the testes were measured using a caliper. To evaluate of histological assay, right testes were fixed in fresh 10% formalin. Every testis was sampled for five vertical sections from the equatorial regions. Ethanol and xylene were used for dehydration step after that each sample were implanted in paraffin; sectioned at thicknesses of five μm and stained with hematoxylin and eosin. Finally our indices were monitored by light microscope.

Spermatids were monitored and evaluated in five circular-transverse sections of testicular tubules. Total, lumen and cellular diameters (μm), lumen, cellular and cross sectional area ($\times 10^4$ μm^2), number of tubules (per 5×5 mm^2) and numerical density were determined in 10 circular transverse sections of different region of testis [21-23].

The mean seminiferous tubule diameter (D) was derived by taking

the average of two diameters, D1 and D2 at right angles. Cross-sectional area (Ac) of the seminiferous tubules was determined using the equation Ac= π (D/2)^2, where π is equivalent to 3.14 and D, the mean diameter of seminiferous tubules. The number of profiles of seminiferous tubules per unit area (NA) was determined using the unbiased counting frame proposed by Gundersen [24]. Numerical density (Nv) of seminiferous tubules was the number of profiles per unit volume and it was using the modified Floderus equation [25] Nv=NA/ (D+T) where, NA is the number of profiles per unit area, D is the mean diameter of the seminiferous tubule and T, the average thickness of the section (μm). The number of spermatids in 10 tubules per testis of both groups was calculated.

2.7. Statistical analysis

The data of stereological indices of seminiferous tubules were subjected to Kolmogorov-Smirnov test of normality and analyzed by independent sample t-test (SPSS for Windows, version 11.5, SPSS Inc, Chicago, Illinois). The *P*-value of less than 0.05 was considered to be statistically significant. Group means and their standard error were reported in the text and graphs (GraphPad Prism version 5.01 for Windows, GraphPad software Inc., San Diego, CA, USA).

3. Results

Testes in both groups presented tubules with thin basement membrane and tunica propria; as well as normal germinal epithelium showing orderly progression from spermatogonia to spermatocytes with groups of spermatids and mature spermatozoa (Figure 1 and 2). Sertoli cells were compressed between the germinal cells and were not easily seen. The interstitium contains normal numbers of Leydig cells.

There was no difference in weights (g) and sizes (length and diameter; mm) between testis in the sham and EMF exposure animals (P>0.05, Figure 3). In stereological analysis, our result showed that lumen diameter (μm) and luminal area (×10^{4}μm^2) of the seminiferous tubules in experimental group were less than the sham group (P=0.02 and P=0.04, respectively; Figure 4A and 4D). However, there were no different between cellular diameter (μm) as well as cellular area (×10^{4}μm^2) in sham and experimental groups (P>0.05, Figure 4B and 4E). Total diameter (μm) and cross sectional area (×10^4 μm^2) of the tubules of the seminiferous tubules in experimental group was less than control group (P=0.004 and P=0.005, respectively; Figure 4C and 4F). Moreover number of seminiferous tubules per unit area (per 5×5 mm^2) of testis in experimental group was more than control group (P=0.03; Figure 4G and 5) and numerical density of the seminiferous tubules in experimental group was more than control group (P=0.005, Figure 4H). However, there were no different in spermatids number of seminiferous tubules between sham and experimental groups (P>0.05, Figure 4I).

In the sperm quality evaluation, percentage of motile sperm in experimental group was less than control group (P=0.02; Figure 6A).

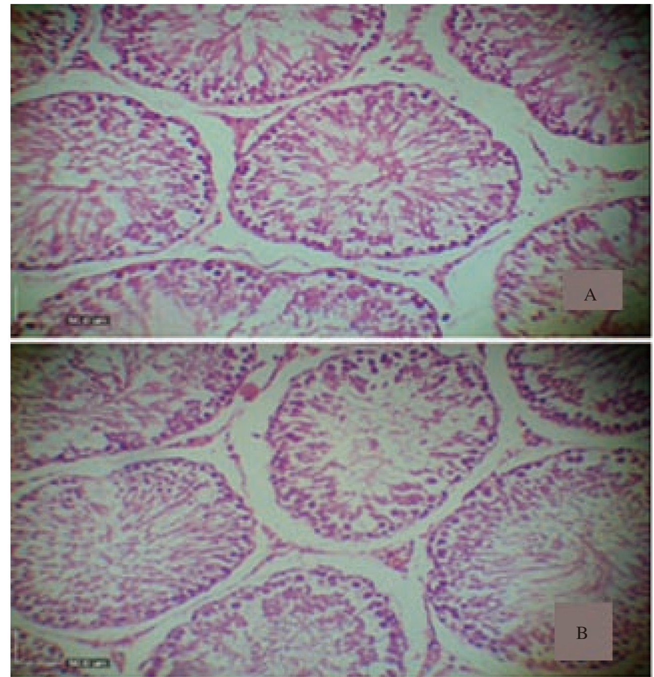

Figure 1. Seminiferous tubules in rats with A, normal and B, electromagnetic field exposed testes. Scale bar is 50 μm (Hematoxylin and eosin staining).

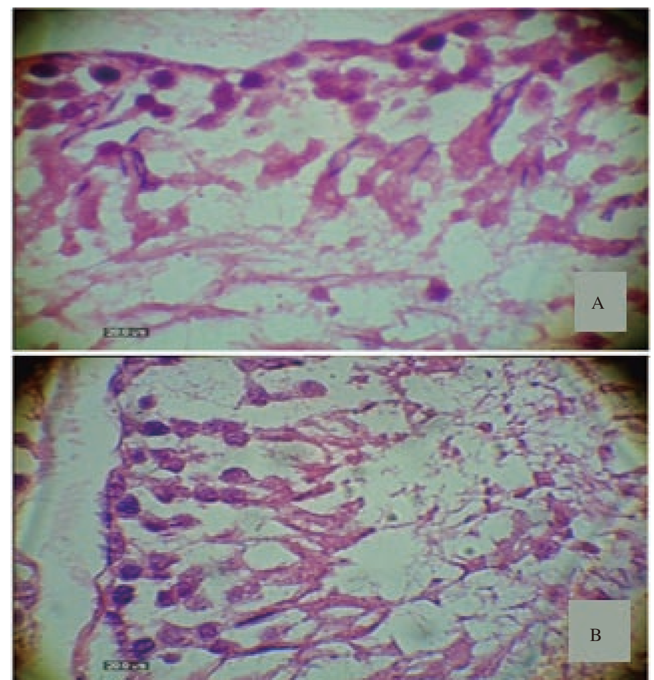

Figure 2. Seminiferous tubules with thin basement membrane and tunica propria; as well as normal germinal epithelium showing orderly progression from spermatogonia to spermatocytes in rats with a, normal and b, electromagnetic field exposed testes.
Scale bar is 20 μm (Hematoxylin and eosin staining).

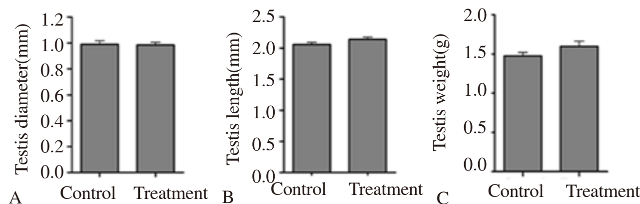

Figure 3. Mean and standard error of a, testis diameter (mm), b, testis length (mm), and c, testis weight (g) in rats with normal and electromagnetic field exposed testes.

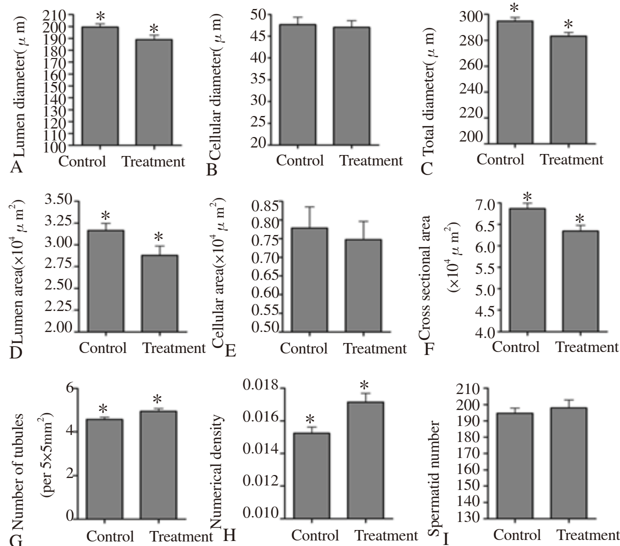

Figure 4. Mean and standard error of stereological indices of seminiferous tubules in rats with normal and electromagnetic field exposed testes. a, lumen diameter (μm), b, cellular diameter (μm), c, total diameters (μm), d, luminal area ($\times10^4$ μm^2), e, cellular area ($\times10^4$ μm^2), f, cross sectional area of the tubule ($\times10^4$ μm^2), g, number of seminiferous tubules per unit area of testis (per 5×5 mm^2), h, numerical density of the seminiferous tubules, and i, spermatids number of seminiferous tubules. Different superscript letters show significant differences between groups.

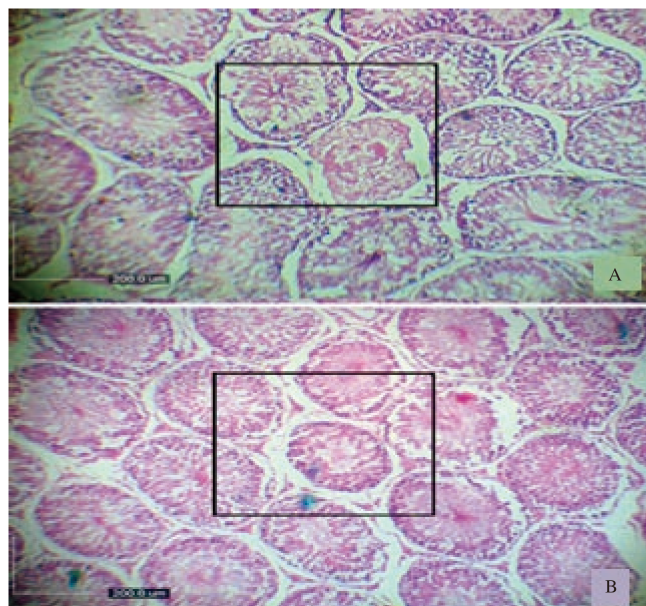

Figure 5. The number of profiles of seminiferous tubules in rats with a, normal and b, electromagnetic field exposed testes per unit area was determined using the unbiased counting frame. Scale bar is 200 μm (Hematoxylin and eosin staining).

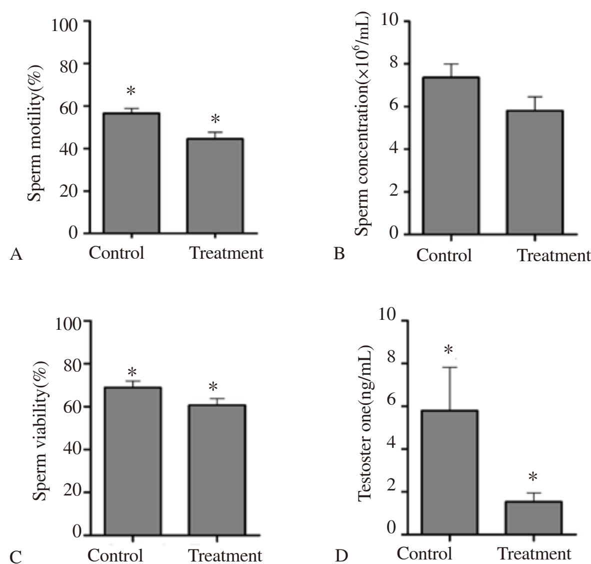

Figure 6. TMean and standard error of sperm quality evaluation indications, a, percentage of sperm motility, b, sperm concentration ($\times10^6$ /mL), c, Percentage of sperm viability, and d, Testosterone levels (ng/mL) in rats with normal and electromagnetic field exposed testes.

Different superscript letters show significant differences between groups.

4. Discussion

Long-term exposure to low frequency EMF decreased the diameter of the seminiferous tubules and also, increased number of seminiferous tubules per unit area (per 5×5 mm^2) of testis. These findings are consistent with the findings of Salama *et al.* [26] who indicated that exposure to 800 or 900 MHz GSM RF radiation (8 h/day for 12 weeks) in standby mode caused a significant decrease in the diameter of seminiferous tubules in the adult rabbit. In addition, Ozguner *et al.* [7] reported that the diameter of the seminiferous tubules and the mean height of the germinal epithelium were significantly decreased in adult EMF-exposed male rats. In contrast, it was reported that electromagnetic radiation induced a significant increase in the diameter of the seminiferous tubules with a disorganized seminiferous tubule sperm cycle interruption of rat [27]. Moreover, Trosic *et al.* [13] reported no significant effect of the applied radiofrequency radiation on testicular histological analysis (testicular function or structure). In addition, radiofrequency radiation emitted from cellular phones has no statistically significant alteration in testicular function or structure[28].

The sperm quality evaluation showed that sperm motility in rats exposed to 50 Hz EMF for 24 h/day for 85 days reduced. However, EMF did not affect the total sperm concentration and viability. Consistent with our findings, cell phone waves decreased sperm parameters in human semen samples [3]. Rats exposed to mobile phone waves for 1 h/day for 28 days showed reduced percentage of motile sperm [5]. Sperm count and motility in Wistar rats decreased

as the magnetic field strength increased [19]. Magnetic fields generated by laptop computers may decrease sperm count and sperm motility in men. Also, sperm count and motility decreased as the magnetic field strength increased[17]. Use of cell phones by men decreased the semen quality by decreasing the sperm count, motility, viability, and normal morphology that was related to the duration of exposure to cell phones[2]. Radiofrequency electromagnetic waves exposure from cell phones adversely affects male fertilizing potential of spermatozoa [29]. Wdowiak *et al.*[4] reported significant harmful effects on male's semen parameters, including motility and morphology because of cell phone usage. Microwave exposure may have a significant effect on reproductive system of male rats, which may be an symptom of male infertility[30]. Exposed to an internet-connected laptop by Wi-Fi for 4 h decreased significantly sperm motility[18]. The prolonged use of cell phones may have negative effects on the human sperm motility and morphology[31]. Exposure to electromagnetic field through cell phones reduced in the human semen quality; including sperm motility and morphology but, does not affect the total sperm count[6]. The spermatozoa in both experimental animals and humans exposed to radiofrequency electromagnetic radiation for the longest time periods decreased motility, concentration, and viability[32]. In contrast to our results, exposure to EMF did not induce any adverse effects on the reproductive capacity including sperm quantity, quality, and morphology[8, 13].

In this study, long-term exposure to low frequency electromagnetic field decreased testosterone levels. Consistent with our results, exposure to static magnetic field (128 mT, 1 h/day for 30 days) decreased rat testosterone levels[8]. Exposure to radiofrequency electromagnetic field decreased testosterone level of male rats[9]. Fifty Hz sinusoidal magnetic field decreased testosterone levels of adult male rats significantly only after 6 and 12 weeks of the exposure period[10]. Long-term exposure to mobile phone radiation leads to decrease in serum testosterone levels. exposed to 30 minutes per day, 5 days a week for 4 weeks to 900 MHz EMF causes significant decrease in serum total testosterone level [7]. In contrast with our findings, exposure to electromagnetic field 1800 and 900 MHz for 2 h continuously per day for 90 days[11] and exposure to 1800 MHz GSM-like[12] caused an increase in testosterone level. However, exposure to circularly polarized, 50 Hz magnetic fields continuously for 6 weeks in rats[14], exposure to static magnetic fields 50 Hz for 40 minutes daily for 17 days[15] and exposure to 50 Hz, 5 mT magnetic field for periods of 1,2 and 4 weeks[16] represented that have no effects on testosterone level of male rats significantly.

In the current study, exposed to low frequency EMF had no effect on the weight and size of testes that is in line with results reported by Amara *et al.* [8]. As well as, Ozguner *et al.* [7] showed that long-term exposure to electromagnetic field emitted from mobile phones has no effect on the weight of rat testes. In conclusion, long-term low frequency EMFs exposure may lead to structural and functional changes of the male testes and may impair male fertility.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

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