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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.
Conclusions: 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 min 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 min 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.

On the other hand, jammer radiofrequency radiation decreased sperm motility in men [17]. In addition, exposed to an

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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 °C, 12-h 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

Rats 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 2000 rpm for 15 min to obtain the serum. Serum was stored at −20 °C 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 °C 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 $Ac = \pi (D/2)^2$, 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