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Long term Exposure to Extremely Low Frequency Electromagnetic Field Affects Sex Hormones Level and Structure of Testis in Rats

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ABSTRACT

Extremely Low Frequency Electromagnetic Fields (ELFEMFs) are produced by a variety of different sources. The aim of this study was to evaluate the influence of ELFEMF on function and structure of testis in rats. Experimental adult male Wistar rats were exposed to a 50 Hz ELFEMF, 1 MT (emitted from solenoid) for 24 h daily during 135 days. The sham rats were subjected to sham exposure and the control rats were kept in animal room. In final, blood samples collected for the determination of the testosterone, LH and FSH concentration in the plasma. The testis was examined using light microscopy. Results showed that in EMF exposed group plasma concentration of testosterone was decreased ($p<0.001$), plasma LH concentration was increased ($p<0.01$) and FSH showed no significant changes which were accompanied by marked atrophy of seminiferous tubules and marked increase in interstitial connective tissue as well as Leydig cell hyperplasia. In conclusion, long term exposure to ELFEMF could have adverse effects on mammalian reproduction.

Key words: Long term exposure, electromagnetic field, testis, testosterone, LH, FSH

INTRODUCTION

Biological effects of exposure to Extremely Low Frequency Electromagnetic Fields (ELFEMFs) have been reported by several authors (Lacy-Hulbert *et al*., 1998; Lazetic *et al*., 1997; Blank and Goodman, 2002; Mostafa *et al*., 2006; Al-Akhras *et al*., 2006; Roushangar and Rad, 2007; Khaki *et al*., 2008; Kilicalp *et al*., 2009; Zamanian *et al*., 2010; Zare *et al*., 2007; Gholampour *et al*., 2011). Since, ELFEMFs are associated with the production, transmission and use of electricity; thus the potential for human exposure is very high (Chung *et al*., 2005). Therefore, the possible adverse effects of ELFEMF on reproduction have been extensively studied in both experiments involving animals and humans over the past several decades (Brent, 1999; Braune *et al*., 2002; Kato *et al*., 1994a; Margonato *et al*., 1993; Marino *et al*., 2001) but the results of them are mostly inconsistent and contradictory.

The production of appropriate numbers of spermatozoa depends upon stimulation of the testes by the gonadotropic hormones, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), both produced by the pituitary gland in response to Gonadotropin Releasing Hormone (GnRH) from the hypothalamus. In response to LH, testosterone is produced by the Leydig cells (Zirkin, 1998). FSH stimulates testicular growth and enhances the production of an androgen-binding protein by the sertoli cells which are a component of the testicular tubule necessary for

sustaining the maturing sperm cell. This androgen-binding protein causes high local concentrations of testosterone near the sperm, an essential factor in the development of normal spermatogenesis (Grover *et al*., 2005).

Since, the possible effects of ELFEMF exposure on male reproductive processes are few and experimental outcomes are quite different, the current study was undertaken to further evaluate the influence of long-term exposure to ELFEMF on functional-morphological features of testis in rat.

MATERIALS AND METHODS

This study was conducted in Shiraz University laboratory in autumn, 2009. EMF exposure unit (at a frequency of 50 Hz and 1 MT) was an open wooden box (100×100×35 cm). The distribution of EMF flux density was measured using a gauss meter.

The research material consisted of 45 male rats of the Wistar strain (234.4±12.6 g). During the experiment, rats were kept in either magnetic field chamber or a similar chamber without a magnetic field or in ordinary cages in the same animal room under controlled temperature of 21-22°C for 135 days. The lighting was turned off or on under a 12 h cycle. The rats were fed with standard granulated feed and had free access to water. The animals were randomly divided into three equal groups of 15 rats each; the groups had no significant differences in body weight. The local ethics committee approved the study.

At the end of experiment animals were weighed, anaesthetized with ethyl ether and blood (average 5 mL) was obtained from the right ventricle of heart and then decanted and centrifuged at 10000 rpm. The plasma was used to determine testosterone, LH and FSH concentrations. After opening the abdominal cavity, left testis was taken which after appropriate fixation and preparation was used for structural examination in light microscope. Plasma concentrations of testosterone, LH and FSH were measured with the radioimmunoassay method (Tabeshyarnoor Co., Hamedan, Iran).

Histopathological examinations: The excised left testis was fixed in the buffered 10% formaldehyde (Merck, USA), embedded in paraffin and 5 μm sections were obtained by microtome (Erma, Japan). Sections were subjected to routine staining with hematoxylin and eosin (H and E).

In a blinded fashion, each section was examined in at least 10 randomly selected non-overlapping fields under light microscope. The testicular histopathology were quantified for the atrophy of seminiferous tubules, increase in interstitial connective tissue and Leydig cell hyperplasia. The level of each pathological manifestation was graded according to the changes involving: none with 0, less than 20% with 1, 20-40% with 2, 40-60% with 3, 60-80% with 4, greater than 80% with 5. The sum of all numerical scores in each group was taken as the total histopathological score (Table 1).

Table 1: Histopathological score of EMF exposed male rats (Mean±SEM)

Groups	Histopathological score
Control	0^{a}
Sham operated	0^{a}
EMF exposed	5^{b}

^{a,b}Means in a column with no common superscript differ significantly ($p<0.05$)

Statistical analysis: The results of plasma testosterone, LH and FSH concentrations, in different groups, are presented as mean values±SEM and were statistically analyzed with the ANOVA test, followed by the *post hoc* Duncan's test. The histopathological scores were statistically compared between groups by non-parametric Kruskal-Wallis multiple comparison test. All data analyses were performed using SPSS ver. 17 software and significance was taken at $p<0.05$.

RESULTS AND DISCUSSION

The main objective of this experiment was to study the effect of long term exposure of adult male rats to ELFEMF (50 Hz and 1 MT) on functional-morphological features of testis.

The seminiferous tubules of control rats had normal size and were full of spermatogenic cells, with scanty interstitial connective tissue and few Leydig cells. The light microscopic examination of the testis sections in the rats exposed to EMF for 135 days revealed marked atrophy of seminiferous tubules and marked increase in interstitial connective tissue as well as Leydig cell hyperplasia (Fig. 1). It is known that testosterone is needed in very high quantities for maintenance of the reproductive tract. Thus, this atrophy of seminiferous tubules might be caused by the decrease in the levels of testosterone observed in this study (Table 2). Also, Leydig cell hyperplasia may be caused as a compensatory response to the reduction of testosterone concentration. In agreement with the current study, Khayyat (2011) found that exposure of mice to EMF caused atrophy in the seminiferous tubules and Rajaei *et al.* (2009) mentioned that exposure to EMF for long periods could decrease the diameter of reproductive ducts. In contradiction to our results Margonato *et al.* (1995) did not find any magnetic field-induced morphologic and histological changes in tested rats after prolonged exposure to a 50 Hz magnetic field at 5 μT.

On the other hand, our results showed the increase in plasma LH concentration after exposure to ELFEMF (Table 2). The significant decrease in testosterone serum level in EMF exposed group may be one of the factors that leads to the significant increase in LH serum level in the exposed group.

LH is known to bind to receptors in Leydig cells and regulate gonadal function by promoting sex steroid production and gametogenesis (Warita *et al.*, 2006). Leydig cells present in the interstitial compartment are the source of testosterone in the testes (Zirkin, 1998). LH is a glycoprotein gonadotropin secreted by the anterior pituitary in response to gonadotropin-releasing hormone (GnRH). GnRH release from the hypothalamus into the portal circulation is episodic which in turn causes LH to be released in a series of secretory bursts, resulting in intermittently elevated LH concentrations in the blood (Bergendahl *et al.*, 1996). LH release is driven mainly by the increase of Ca^{2+}. ELFEMF may modulate cellular calcium regulatory mechanism which affects the affinity of calcium binding proteins such as calmodulins. The modulatory effect of calcium may affect pituitary genital axis (Mostafa *et al.*, 2006; Lacy-Hulbert *et al.*, 1998). In addition, it has been shown that melatonin inhibits GnRH induced increase in intracellular concentrations of cAMP and calcium (Vanecek and Klein, 1992; Vanecek, 1998). Thus, melatonin induced decrease of cAMP

Table 2: Mean plasma concentration of testosterone, LH and FSH of EMF exposed male rats (Mean±SEM)

Groups	Testosterone (ng mL^{-1})	LH (ng mL^{-1})	FSH (ng mL^{-1})
Control	1.70±0.09[a]	1.66±0.11[a]	2.58±0.09[a]
Sham operated	1.80±0.09[a]	1.56±0.13[a]	2.63±0.12[a]
EMF exposed	0.29±0.06[b]	2.28±0.15[b]	2.51±0.11[a]

[a,b]Means in a column with no common superscript differ significantly ($p<0.05$)

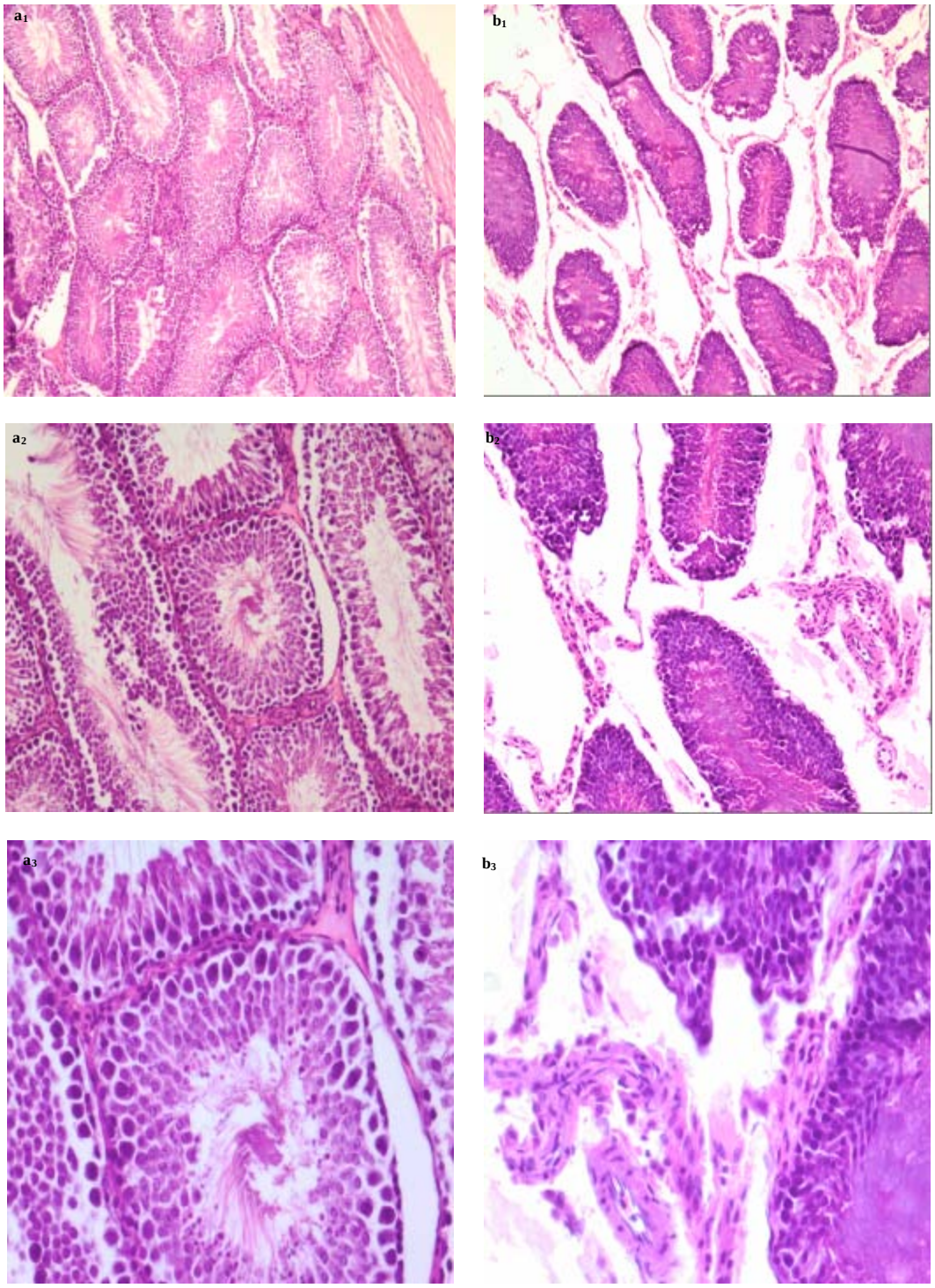

Fig. 1(a-b): (a_1, a_2 and a_3) Representative light microphotographs of the testis obtained from the control group, (b_1, b_2 and b_3) EMF exposed group, (H and E staining; a_1 and b_1; x100; a_2 and b_2; x200, a_3 and b_3; x400)

may affect the LH release via inhibition of GnRH induced calcium increase. On the other hand, ELFEMF have been reported to inhibit nocturnal production of melatonin in rats (Kato *et al.*, 1994b; Kumlin *et al.*, 2005) and in Hamsters (Yellon, 1994). Therefore, the significant increase in plasma LH levels in ELFEMF exposed group may be explained on the basis of inhibition of nocturnal production of melatonin in these rats.

Contradictory to present results Margonato *et al.* (1993) did not find any differences on LH and testosterone between exposed animals and control group's animals after exposure to high intensity electric field for up to 18% of their life span.

In our finding, absence of significant changes in the level of FSH hormone in rats exposed to ELFEMF for 135 days is in harmony with the finding of Al-Akhras *et al.* (2006), they reported that there were no significant effects on the serum's level of male Follicle Stimulating Hormone (FSH) during the 18 weeks of exposure period. But, Free *et al.* (1981) have found alterations in the secretion pattern of FSH in rats exposed to an 80 KV m^{-1} electric field for 20-56 days.

CONCLUSION

These results suggest that long-term exposure to ELFEMF can alter hypothalamic-pituitary-gonadal hormones and produces histopathological effects on the testis of rats.

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