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Histological Study of Testes and Sperm Parameters in Adult Mice Exposed to 50 Hz Electromagnetic Field during Developmental Period

Bushra A. Hamdi, [1] Leila Roshangar,[2] Amir A. Khaki, [2] Jafar Soleimani-Rad*[2]

[1]Clinical Analysis Department, Pharmacy college, Hawler Medical University, Iraq, Kurdistan - Region
[2]Anatomical Sciences Department, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz- Iran

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ABSTRACT

Detrimental effect of Electromagnetic Field (EMF) on reproductive system has been of critical concern for a long time. The aim of the present study was to evaluate the effect of EMF exposure during developmental period on histophysiology of testis in adulthood. For this purpose the adult male and female Balb/c mice were mated and pregnant mice were divided into control and experimental groups. In the experimental group, the pregnant mice were exposed to 3 mT EMF, 4 hours /day during pregnancy. The control group were treated similar to experimental group but without exposure to EMF. After delivery, both in control and experimental groups the male pups were kept under normal condition till reach adulthood. Then they were sacrificed and their testes were fixed and paraffin sections were stained and examined with light microscope. Microscopy revealed that in the seminiferous tubules from experimental group several intercellular spaces were present and spermatogenic cells appeared to be disrupted. The nuclei of the different spermatogenic cells were dense and hyperchromatic. Evaluation of semen parameters showed that the concentration of sperms and their motility were decreased significantly in comparison to control group. Furthermore, morphological abnormalities were increased significantly in experimental group in comparison to control group. In general, it is concluded that EMF exposure during intrauterine life could affect the semen quality and spermatogenesis in adulthood which may lead in subfertility.

Key words: Spermatogenesis, Electromagnetic Field, sperm motility, sperm morphology.

INTRODUCTION

Nowadays it is a well known fact that electromagnetic field (EMF) can be detected everywhere on the earth, regardless of its being natural or manmade. During last three decades, the application and the use of equipments related to electrical energy have tremendously increased all over the world (1, 2, 3). Attention has been drawn to the biological effects of electromagnetic

fields (EMF) in general particularly on reproductive system (4). Reviewing the literature on the effects of EMF reveals that the results on the effects of EMF on living animals are contradictory. Scientific research have shown that rats exposure to EMF during developmental period, on 13.5 days post-conception, may affect testicular structure and fetal development (1, 5, 6).

In contrast, a number of studies showed that exposure to EMF do not produce any detectable alterations in the offspring's, spermatogenesis and fertility in animals and human (7, 8, 9). Where as, some other studies conducted by other scientists showed clear damage to spermatogenesis (10, 11, 12, 13). It has been reported that exposure of fractionated doses of magnetic fields (20 mT) caused a significant decrease in sperm count, motility, daily sperm production and changes in testicular components.
several reports have shown that EMF generally decreases sperm motility, and the tail abnormality could be the outcome of the effects of exposure to EMF (14, 15, 16). In addition to acute adverse effects of electromagnetic radiation on sperm motility, long-term Electromagnetic Radiation exposure may lead to behavioral or structural changes of male germ cell (8, 12, 17).

Nevertheless, studies using low EMF intensity showed that rat exposed to 50 Hz magnetic field during 8-15 days of development did not show any observable effects on reproduction and embryo-fetal development (18). Where as other study (19) reported that after EMF exposure, there was not a significant difference in sperm parameters including: morphology, number and motility of sperms. There was also no difference in fertility and pregnancy ratio. The effects of 60 Hz magnetic fields on rats showed no biologically significant effects on reproductive performance (20). In contrast, some study showed that exposure of adult rats to 50 Hz magnetic field for 90 days had some adverse effects such as reduced fertility of male and female rats (21). It is also shown that exposure to EMF has some detrimental effect on the germ cells which depend on exposure time, proliferation and differentiation of spermatogonia and these effects include: significant decrease in sperm number, motility and the histological structure of the testis (22, 23, 24, 25).

The present study was carried out to investigate the effect of 50 Hz EMF exposure during developmental period on testicular structure and sperm parameters, such as: motility and morphology of sperm during adulthood.

MATERIALS AND METHODS

Animals: The animals that were used in this project were adult male and female Balb/c mice (9-10 weeks old) weighing 25-35 g. The mice obtained from animal house unit in Department of Histology, Faculty of Medicine, Tabriz university of Medical sciences, Tabriz-Iran.

The mice were kept in a room with 12 h light/ 12h dark photoperiod, room temperature (20°-22° C) and relative humidity of 50-60% during the exposure period.

For mating, one male and two female mice were housed in a cage and observation of vaginal plug, on the next morning, was designated as 0 day of pregnancy. The pregnant Mice were divided into two groups, each with 15 pregnant mice. The experimental group was exposed to 3 mT (50Hz) magnetic field in the EMF producing device, for cage for 21 days, 4 hours /day. The exposure time was from 8:00 am to 12:00 noon. While the other group (control) were kept in the same condition but without exposure to EMF. After delivery, the male pups from both groups were kept under normal condition till to reach adulthood. Then, they were sacrificed by cervical dislocation and their testis were removed and prepared for histological studies.

Histological studies

The testis were dissected apart, cut into two pieces and were immediately fixed in alcoholic Bouins solution for 24 hours, then dehydrated and finally embedded in paraffin and were sectioned serially in 5 mm thick sections. The sections were stained with hematoxylin and eosin (H&E) and studied with the light microscope.

Study of sperm parameters

Sperm were collected from caudae epididymides. After killing the mice, vasa deferentia were removed and placed in a plastic plate containing 1 ML Ham's F-10 medium (prewarmed to 37° C, with the precaution of removing of fat pads surrounding the tissues before sperm collection). Then the cauda epididymides were cut into small pieces and transfered to Co2 incubator for 20 min. Counting of sperms, was performed as high per field (HPF) using 40X objective and then the concentration and motility of sperms was calculated as million per milliliter (M/ml). Evaluation of sperm's morphology was carried out in the same manner but calculated as percentage.

Statistical analysis

The data were analyzed and compared with control group using (student t-test). The p values < 0.05 was considered as significant through out this study.

RESULTS

Light microscopic examination showed that in the control group, testes had a normal testicular architecture with an orderly arranged spermatogenic and sertoli cells. The spermatogonia and sertoli cells were rested on the basement membrane of the seminiferous tubules. The Leydig cells with large and acidophilic cytoplasm were located in the interstitial tissue among seminiferous tubules (Fig. 1).

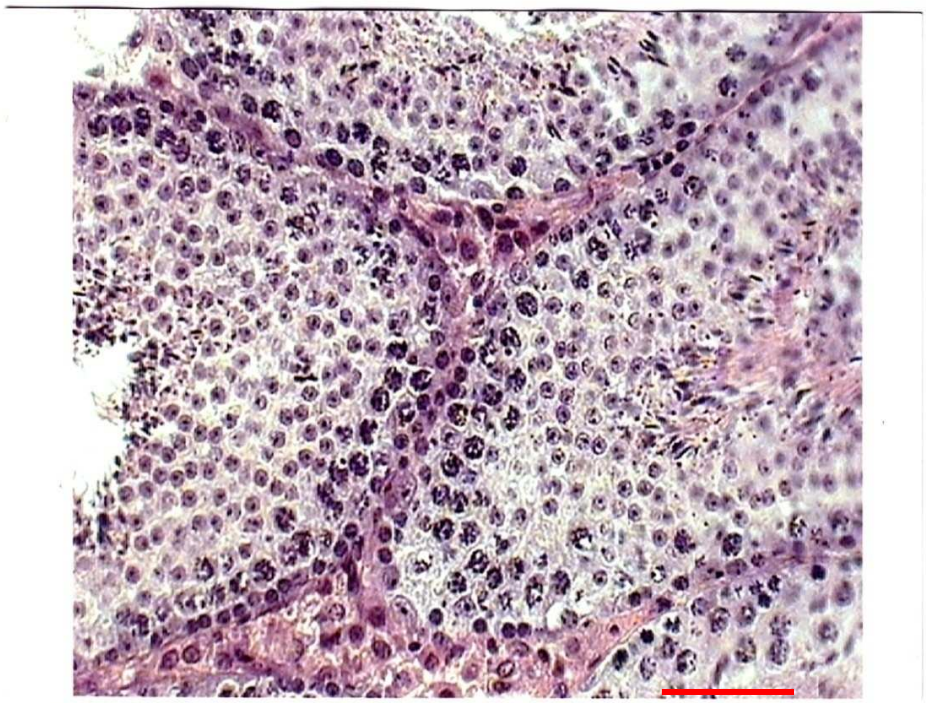

Figure 1: Light microscopic micrograph of seminiferous tubules from adult mice in control group. Note normal spermatognic epithelium composed of different spermatogenic cells, sertoli cells (T), leydig cells (L). H&E staining. Scale bar=53 μm

In the experimental group the spermatogenic cells in the germinal epithelium were separated from each other by numerous empty spaces (Fig. 2). Another prominent finding in the experimental group, in comparison to control group, was condensation of the nuclei of dividing cells (Fig. 2). Mature spermatozoa was rarely present and only few tubules contained mature spermatozoa. Many atypical tubules in the various stages of sperm development were found in exposed group that showed disorganization and or loss of spermatogenic epithelium. A large number of germ cells were also found in the lumen of seminiferous tubules (Fig. 2). The sertoli cells appeared not to be affected but some sertoli cells were flattened (Fig. 2). The Leydig cells in the interstitial spaces had a normal morphology.

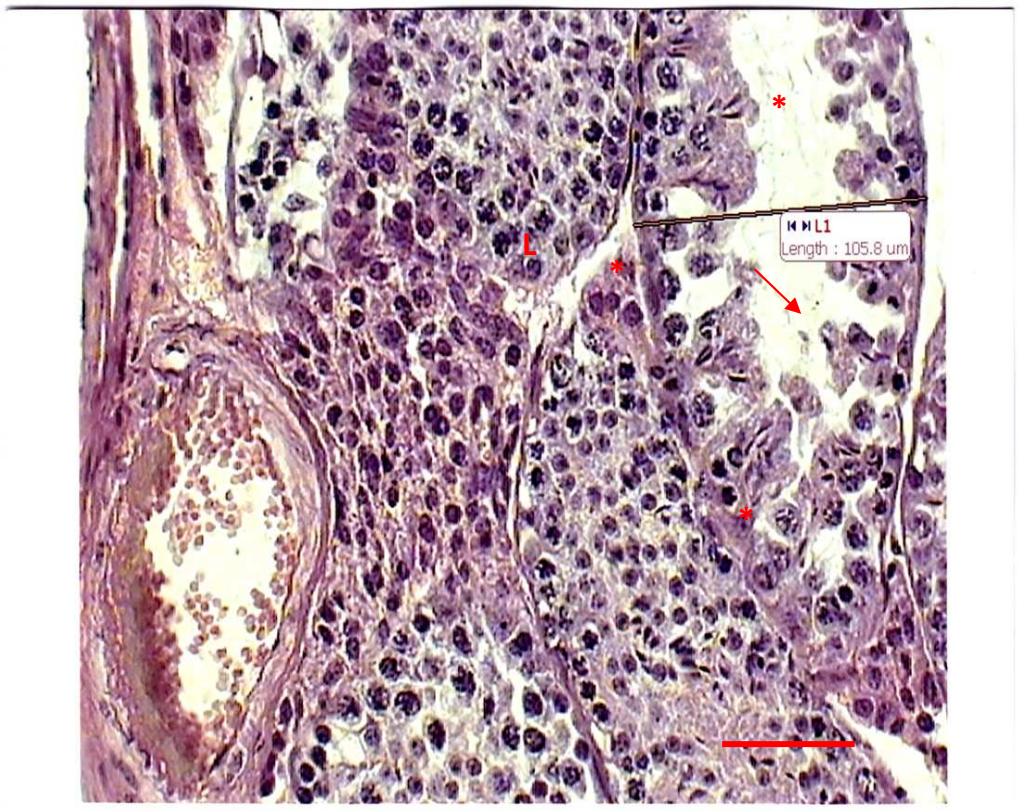

Figure 2: Light Microscopic micrograph of semniferous tubules from exposed mouse. Note intercellular spaces (*). Elongated spermatids and mature sperms are rare, Leydig cells (L). Flattened sertoli cells (→) (H&E) staining. Scale bar= 53 μm

Morphological evaluation of sperms revealed that defective sperms such as double headed sperms, double tail sperms, headless sperms, tailless sperms, sperms with large or small head, and sperms with crocked neck were present in both groups but appeared to be more frequent in EMF exposed group (Fig. 3).

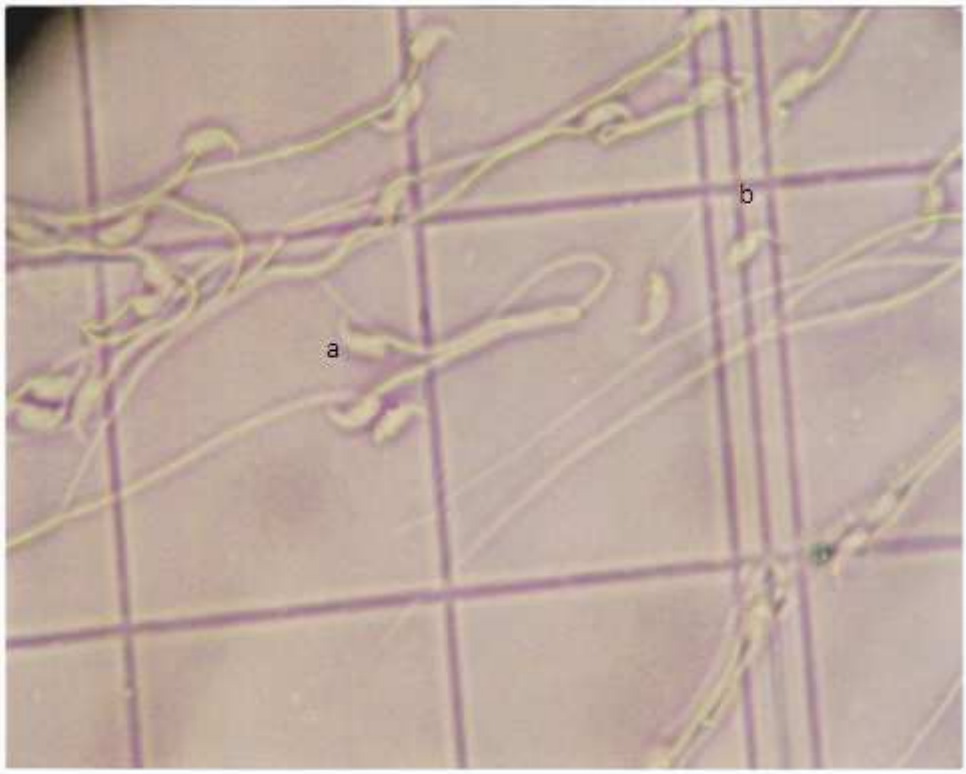

Figure 3: Types of sperm abnormalities in EMF exposed mice.forms. Sperm with tow head (a), sperm without tail (b).

Table 1 summarizes the morphological abnormalities considered in both control and exposed groups. As it is shown in the table, the percentage of abnormalities such as; double tail sperms, tailless sperms, sperms with defective head, and sperms with cytoplasmic droplets were significantly higher in exposed group in contrast to the control (P <0.005). However, the frequency of other abnormalities including; double head sperms and headless sperms were similar in control and experimental groups.

Table 1: Comparison of sperm abnormalities in control and EMF-exposed groups (as percentage).

	Control group (10 mice)	**EMF exposed group (10 mice)**	
Types of abnormality	**Mean ± S.D**	**Mean ± S.D**	**p-value**
Double tail sperm	None	6.00 ± 0.1	0.001
Tailless sperms	0.90 ± 0.2	1.20 ± 0.3	0.023
Defective head sperm	0.62 ± 0.5	1.30 ± 0.4	0.004
Double head sperm	None	0.10 ± 0.02	0.160
Headless sperms	1.26 ± 0.16	1.32 ± 0.1	0.465
Sperms with cytoplasmic droplet	1.24 ± 0.2	3.72 ± 0.9	0.001

P<0.05 is considered as significant

Table 2 summarizes the effect of EMF exposure on sperm concentration and motility. As it is shown in the table, the total number of sperms in EMF-exposed group decreased significantly (P <0.001) in comparison to control group (19.4 ± 1.55 M/ml vs 22.4 ± 2.15 M/ml). Accordingly, the number of motile and progressive sperms were also decreased significantly (P< 0.001). Comparison of proportion of motile and progressive sperms revealed that in control group 60% of sperms were motile in which 35% were progressive. In experimental group, in contrast, 50% of sperms were motile and only 25% of them were progressive. Both motile sperms and sperms with progressive motility were significantly decreased in EMF-exposed group (P<0.01).

Table 2: Sperm concentration and motility in control and experimental groups, as million per milliliter (M/ml).

	Control group	Experimental group	
	Mean ± S.D	Mean ± S.D	p – value
Sperm concentration	25.76±2.15 M/ml	19.58± 1.55 M/ml	0.001
Motile sperm	15.45±1.45 M/ml (60%)	9.72 ± 1.11(50%)	0.001
Progressive motility	5.4±0.98 M/ml (35%)	2.54 ± 0.69 (25%)	0.001

DISCUSSION

In the present study the effect of EMF exposure during developmental period on spermatogenesis in adulthood is investigated. The results showed that EMF exposure have a detrimental effect on architecture of germinal epithelium in the seminiferous tubules (Figs 1-2) and significantly affect sperm parameters (tables 1-2).

Conflicting observations have been reported regarding the potential toxic effects of EMF on reproduction and spermatogenesis in experimental animals (10, 26, 27, 28, 29).

The exposure of adult male rats to electromagnetic field may lead to reduction in their fertility, increasing of sperm morphological abnormality, and decreasing of their motility and concentration (9, 27, 28, 30, 16, 31, 14). Our results show that exposure to EMF, during developmental period, could affect sperm parameters on the adulthood. This finding indicates that EMF exposure, not only could affect the developing testis but its toxic effects are irreversible, which is the main point of our study.

Several authors reported adverse effects of electric and magnetic fields in relation to fetal development and the male reproductive system (32, 33). Exposure of rats to 60 Hz and 1mT EMF, from the 13^{th} day of gestation to the 21^{st} postnatal day caused delayed testicular development (6). It is also shown that EMF exposure could induce apoptosis in spermatogenic epithelial cells (12, 30).

In this study, presence of cells with dense and hyperchromatic nuclei and their separation from neighboring cells were evident (Fig. 2). These features are considered as pre-apoptotic signs and indicate that apoptosis induction not only occurs at exposure time, but also continuous for a longer period after exposure. Genetic effect of EMF exposure could explain this phenomenon. The effect of EMF on DNA and the involvement of genes even in the morphology of normal sperms have already been described (14, 34).

In contest there have been several articles addressing the effects of radio frequency and higher frequency EMF on spermatogenesis in animal models. They have shown that there were not any effect on apoptosis induction, sperm motility and spermatogenesis disorder (25, 35, 36, 24).

The finding that electromagnetic field exposure cause nuclear condensation and marginal hyper chromatin of germinal epithelium has also shown in previous studies (15, 37, 38). The effect of EMF on different morphological categories such as defective head sperms, double head sperms, double tail sperm, and sperm motility, whether it is progressive or non progressive, were evident throughout the present investigation. Our results are in agreement with the findings of Bernabo, et al., 2006, that EMF do has effect on sperm motility. The present results show that exposure of developing mice to EMF for 21 days had significant effect on development of testis and its

function during adulthood. In accordance with our findings developmental effect of EMF as a detrimental effect on fetal growth, miscarriage, occurrence of malformations such as polydactylia, fused rib, curled tail and brain hernia has already been reported (39, 40, 2, 41, 42). The findings document the effect of exposure, particularly in respect to condensation of nuclei and appearing of large irregular spaces indicate a gap of knowledge regarding the effect of EMF on reproduction process in animals: it should be filled up in years to come in order to confirm the effect of EMF on animals and human.

CONCLUSION

The results of the present investigation demonstrate that exposure to 50 HZ /3 mT EMF during embryonic life, adversely affect the structure and function of testis in adulthood. Indicating that detrimental effect of EMF on developmental period is irreversible and may lead to subfertility or infertility in adulthood.

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