



The Effects of 50 Hz Electromagnetic Fields Induction of Apoptosis in Rat Ovarian Follicles

Seyed Shahin Ahmadi¹, Amir Afshin Khaki^{1*}, Alireza Alihemmati², Asghar Rajabzadeh³, Giti Seyed Giasi⁴

Abstract

Objective: The increases application of electromagnetic fields (EMFs) equipments in last decades led to some diseases. Therefore, many studies focused in this issue mainly on critical organs like central nervous system and reproductive function. In the meantime, female infertility by EMFs is one of the most important challenges in current studies.

Materials and Methods: Thirty rats were selected randomly and divided into 3 groups (n = 10). Group 1 received 50 Hz EMFs for 8 weeks (3 weeks via intrauterine + 5 weeks after births). Group 2 received 50 Hz EMFs for 13 weeks (3 weeks via intrauterine + 10 weeks after birth). Group 3 was considered as a control in the normal condition. After this period, evaluation apoptosis in ovarian follicles were analyzed by TUNEL method.

Results: The results of this study show a significantly increased of apoptotic cell members in experimental groups. Degenerative and pathologic changes were found in ovarian tissue and follicles besides which indicates increase the number of apoptotic cells in experimental groups. As well as, this study has shown that oocyte nucleus was smaller and deformed and apoptotic vacuoles increased in granulose cell.

Conclusion: According to the results, we observed that EMFs can changes oogenesis cycle through destructive impacts on ovary structure and function. Of course, this problem depends on frequency and duration of exposure to these waves.

Keywords: Electromagnetic fields, Apoptosis, Rat follicle

Introduction

Electromagnetic radiations which are briefly called EM radiation or electromagnetic field (EMF) are Emission waves phenomenon's that spreads in vacuum or Article. These waves consist of electric and magnetic fields that are perpendicular to the direction of energy. According to frequency, electromagnetic radiation are classified into radio waves, radar waves, infrared radiation, visible light, ultraviolet, X-rays and gamma (1). This waves are widely used in daily life devices and equipment such as refrigerators, freezers, televisions, radios, microwave ovens, photocopiers, computer monitors, printers and halogen lamps (2). The frequency and wavelength rang of microwaves is variable from 300 MHz to 300 GHz and 1 mm to 1 m, respectively (3). Also, emitted waves of cell phones with frequency is about 900 MHz to 1 GHz (4). With radiation of these waves on molecules, its energy absorbed by molecules that causes changes in molecular vibration or temperature. The biological effects of microwave radiations are complex and controversial, but there is evidence which shows different biological effects on Irradiated molecules (5). Increasing use of electrical devices especially mobile phones led to teratogenic

effects on different developmental processes and human health. Non-thermal biological effects can changes in cell functions, proliferation rate and gene mutations that cause cell death (6).

Health and hygiene standard for exposure in EMFs at about 2.5 MHz, has been accepted as universally standard and EMFs higher than this value, are dangerous (7). In recent years, more than 50 studies have been done to study the effects of radiation on different animals (8). The results of studies revealed the increasing of hyperploidy because of chemical-induced in mammalian oocytes (9), reduction in fertility (10), disordering in spermatogenesis (11,12) and also reduction in number of viable embryos in mice (13,14). The effects of EMFs on cell proliferation (15) and apoptosis (16,17) have investigated.

Exposure to electromagnetic with a 50 Hz frequency caused to damages in the basal cells, epithelial nuclei dense and heterochromatin. Also, in the stromal connective tissue was observed abnormal process. It seems that the results of animal and epidemiologic studies can creating strong connection between EMF and public health (18). The findings showed that not significantly changes in the weight and size of ovary and their offspring with expose

Received 19 March 2016, Accepted 12 September 2016, Available online 24 September 2016

¹Tabriz Health Services Management Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. ²Department of Histology and Embryology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran. ³PhD of Anatomical Sciences, Lorestan University of Medical Sciences, Khorramabad, Iran. ⁴Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

*Corresponding Author: Amir Afshin Khaki, Tel: +989144157161, Email: dr.aakhaki@yahoo.com



to the low frequency EMF. The study of micrographs shows hetero-chromatinated oocytes and follicular cells increased polysomes, accumulation of mitochondria and cleft nucleus. Reduction level of FSH, LH hormones and mating rate was observed compared to control group (19). As well as, some of the studies showed that exposure to EMFs can increase degenerative changes in oocyte nest and follicular formation (20).

The ovaries are located in the abdominal cavity; each ovary contains a large number of bags or cavities called follicles. Thus, with each follicle enlargement, various stages of oogenesis (egg formation) take place within them and after the sexual cells reached into second type of oocytes, follicles open and the second release of the oocyte. And after the sexual cells reach into second type oocyte level, follicle open, rip surface of ovarian and make free the second type oocyte. This cells with follicular fluid go from ovary to ovum egression part. This phenomenon is called ovulation. The final stage of ovum forming (it means the alteration of second type oocyte to ovum) takes place outside the ovary and in vagina (21). This study was aimed to investigate apoptosis on ovarian follicles under EMF condition.

Materials and Methods

Thirty rats were selected randomly and divided into 3 groups (n = 10). Group 1 received 50 Hz EMFs for 8 weeks (3 weeks via intrauterine + 5 weeks after births). Group 2 received 50 Hz EMFs for 13 weeks (3 weeks via intrauterine + 10 weeks after birth). Group 3 was considered as a control in the normal condition. After this period, evaluation apoptosis in ovarian follicles were analyzed by TUNEL method (Roche, In Situ Cell Death Detection Kit, Fluorescein). A: Embedding has done by xylol and paraffin. B: Putting the samples in the microwave equipment 700 W for 10 minutes. C: Incubation of the samples for 60 minutes. D: Washing the samples 3 times in antifluorescein-pod for 30 minutes. G: Washing the samples 3 times in PBS. H: Putting the samples in H₂O₂-Diaminobenzidine. E: Lightly counter-stain tissue sections with hematoxylin stain (Hematoxylin counter-stain) (Sigma-Aldrich; St. Louis, MO). To evaluate of ovarian follicles by light microscopic, all species of tissue fixed at 10% formalin, then they were processed to prepare 5-mm-thick paraffin sections for light microscopy images. Percentage of apoptotic cells in follicles and Granulosa had determined by counting of cells (×40).

Statistical Analysis

Statistical analyses were done in control and experimental groups by SPSS and ANOVA tests. $P < 0.05$ used as minimal of the significant deviation.

Results

This study suggests degenerative and pathologic changes of the ovarian tissue and follicles in groups under EMF

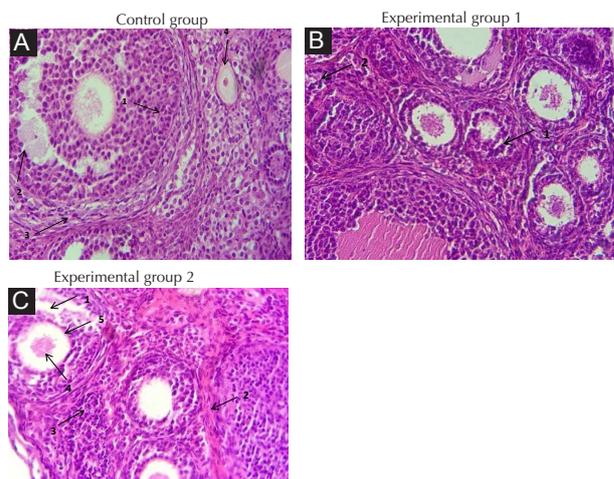


Figure 1. (A) Micrograph of ovarian follicle of control group showed secondarily follicle with obvious granulosa cell (1), antral space (2), internal theca (3) and primordial follicle (4). The view of structures is normal (H&E - ×40). (B) Micrograph of ovarian follicle of experimental group 1 showed growth of follicle with degenerative view in granulosa cells (1) and disarrange of tissue structures, the cell layers and nucleus accumulation of collagen fibers (2) (H&E - ×40). (C) Micrograph of ovarian follicle of experimental group 2 showed growth of follicle with sever degenerative view in granulosa cells (1) and disarrange of tissue structures, the cell layer and nucleus accumulation of collagen fibers (2). Also it showed sever infiltration of leucocytes (3), degeneration of oocyte cytoplasm (4) and zona pellucida (5) (H&E - ×40X).

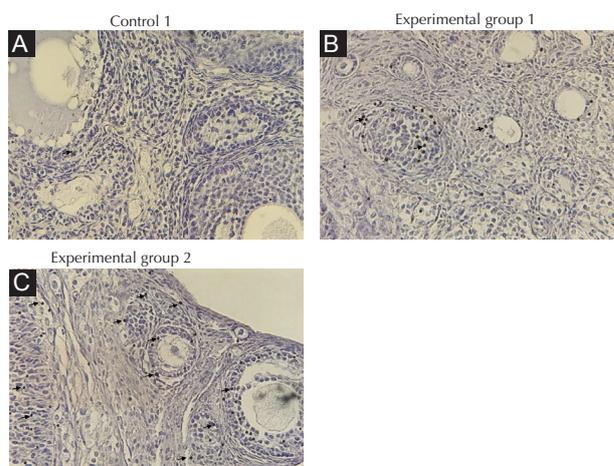


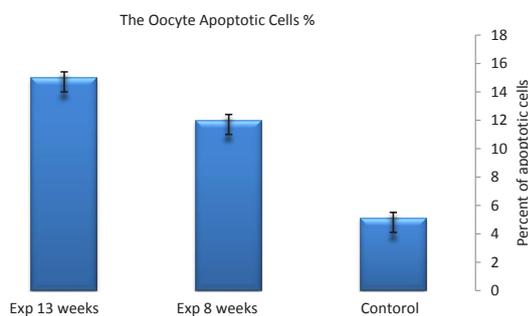
Figure 2. (A) Photomicrographs of ovarian follicles in control group show of granulosa cells with very low tunnel positive (arrow) apoptotic cells. (Tunnel stain and background with hematoxylin stain - ×40). (B) Photomicrographs of ovarian follicles (primary multi layer follicles) in 8 weeks EMF exposed group: The numerous granulosa cells are tunnel positive (arrow) in compare to control group. (Tunnel stain and background with hematoxylin stain - ×40). (C) Photomicrographs of ovarian follicles (primary multilayer follicles & antral follicles) in 13 weeks EMF exposed group: The numerous granulosa and theca cells are tunnel positive (arrow) compared to EMF exposed (8 weeks) group. (Tunnel stain and background with hematoxylin stain - ×40).

besides the increase in number of apoptotic cell in experimental group under EMF exposure. The results of experimental 13 weeks under EMF expose indicated significant increase apoptotic cells relation control group

Table 1. The Granulosa Cells Apoptotic Cells in Different Groups Affected by EMF

Groups	Control	Experimental 8 Weeks	Experimental 13 Weeks
The apoptotic cells in terms %	5.1 ± 0.41	12 ± 0.41*	15 ± 0.41*

*It shows a significant increased of apoptotic cells in experimental groups.

**Figure 3.** Percentage of Apoptotic Cells in Follicles.

(Mean ± SE: 15 ± 0.41) (Figure 1). Also experimental 8 weeks groups under EMF expose indicated significant decrease apoptotic cells experimental 13 weeks group (Figure 2) (Mean ± SE: 12 ± 0.41). Totally, number of apoptotic cells in experimental groups (8, 13 weeks) under EMF expose compare to control group increased (Table 1; Figure 3) $P < 0.05$).

Discussion

Our world is full of a variety of electrical and electronic appliances. Although these devices have made our daily lives easier but secretly causes harm to our health. Electrical devices such as TV, CM, washing machines, mobiles microwave and many electrical appliances such as transmitters and antennas around homes emit dangerous waves which are harmful to human health. Scientist's studies show that long-term presence with these danger waves cause a variety of symptoms, cell death, infertility, DNA damaging, tumor and cancer. In addition to, the radiation waves for pregnant women, fetuses cells and children health have a serious injury (2-5). Studies have shown that EMFs can have devastating effects on the cell and tissue of testicular, spermatogenesis, level proteins of serum and activity of alkaline phosphatase (7,8). EMFs are doing their effects in different ways including changes in the emissions of the membrane, the destruction of connective bands, changes in protein expression, imperial synthesis of macromolecules and changes in nucleotides bases (22,23). The risks of EMFs on reproduction process to forming reduced fertility, implantation rats, reduced of live birth and the incidence congenital malformation have been reported (24-27).

Exposure to EMFs can cause morphological changes in the epithelium of uterus and fallopian tube. It also increases the density of nuclei granulose cells, morphological changes in oocytes and the number of atretic and cystic follicles (28). Accruing to some studies, follicular atresia is caused by apoptosis and activation of certain genes (29-31). In 1977 Mailhes et al showed that

radiation on animals caused an increase in hyperploids of mammalian oocyte (32). Several studies showed that radiation impairs the spermatogenesis (32-24). Also, the study of Al-Akhras et al showed that 50 HZ frequency of EMFs led to significant decreases in the sperms number of rats. In addition, serum levels of FSH and testosterone decrease, while levels of LH increase (35). Hjollund et al reported that the waves do not have a significant effect on hormones (36). Huuskonen et al did not find any change in the estradiol and progesterone levels by using 130 µT EMFs (37). In study conducted by Bakacaka et al, they did not observe significant reduction in the follicles number of rat under exposed with EMFs. The researchers in previous experiments showed that EMFs reduce the follicles number, DNA damage and cause apoptosis and oxidation stress in ovary. Research on the EMFs effects have showed that ovaries nucleuses are smaller and the number of autophagy vacuoles in granulosa cell increased. (38) Morphological changes in the ovary indicate that the cytotoxic effect of EMFs and changes in the granulosa cell is at the same time apoptosis in the granulosa cell. The findings suggest that exposure with EMFs may disrupt the normal process of follicles (39-41). our study indicated EMF expose increased apoptotic cells in ovarian follicles.

Acknowledgments

Authors want to thank from Research Vice-Chancellor of Tabriz University of Medical Sciences for financial support of this study. The authors also thank all family members of participants and the staff in the health centers of Tabriz District Health Center. This paper is based on a PhD thesis (number 5/77/4031) submitted to Tabriz Health Services Management Research Center.

Ethical Issues

This study has been approved by the ethical committee of Tabriz University of Medical Sciences, Tabriz, Iran on date 2012/11/22 (number 5/4/7044).

Conflicts of Interests

None.

Financial Support

Tabriz University of Medical Sciences supported the study.

References

1. Serway RA, Ujewett JW. Physics for Scientists and Engineers. 6thed. Brooks Cole; 2004.
2. Dimitris J, Panagoulos H, Margaritis P. Effects of electromagnetic field on the reproductive capacity of *Drosophila melanogaster* in biological effects of

- electromagnetic fields, mechanism modelin, biological effects. New York; 2003;438-452.
3. Banik S, Bandyopadhyay S, Ganguly S. Bioeffects of microwave brief review. *Bioresour Technol.* 2003;87(2):155-9.
 4. Hyland GJ. Physics and biology of mobile telephone. *Lancet.* 2000;356(9244):1833-6.
 5. Baharara J, OryanSh, Ashraf A. Effects of microwave (940 MHZ) on ovary and fertility of female Balb/C. *Journal of Tarbiat Moallem University.* 2007;7:931-940.
 6. Nakamura H, Matsuzaki I. Nonthermal effects of mobile phone frequency microwave on uteroplacental functions in pregnant rat. *Reprod Toxcol.* 2003;17:321-326.
 7. Adey WR. Biological effects of electromagnetic fields. *J Cell Biochem* 1993;51:410-6.
 8. Robert E. Teratogen update: electromagnetic fields. *Teratology.* 1996;54:305-13.
 9. Repacholi MH. Low level exposure to radiofrequency electromagnetic field: health effects and research needs. *Bioelectromagnetics.* 1998;19(1):1-19.
 10. Zimmermann B, Hentschel D. Effect of static magnetic field (3.5) on the reproductive behavior of mice, on the embryo and fetal development and on selected hematologic parameters (German). *Digitale Bilddiagn* 1987;7:155-161.
 11. Klug S, Hetscher M, Giles S, Kohlsmann S, Kramer K. The lack of effects of nonthermal RF electromagnetic fields on the development of rat embryos grown in culture. *Life Sci* 1997;61:1789-1802.
 12. Cao YN, Zhang Y, Liu Y. Effects of exposure to extremely low frequency electromagnetic fields on reproduction of female mice and development of offsprings (Chinese). *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi.* 2006;24:468-470.
 13. Soeradi O, Tadjudin MK. Congenital anomalies in the offspring of rats after exposure of the testis to an electrostatic field. *Int J Androl* 1989;9:152-160.
 14. Tablado L, Prez-Sanchez F. Effects of exposure to static magnetic fields on the morphology and morphometry of mouse epididymal sperm. *Bioelectromagnetics* 1998;19:377-383.
 15. Mevissen M, Buntenkotter S, Loscher W. Effects of static and time-varying (50-Hz) magnetic fields on reproduction and fetal development in rats. *Teratology* 1994;50:229-37.
 16. Ruiz Gomez MJ, De la Pena L, Pastor JM, Martinez Morillo M, Gil L. 25 Hz electromagnetic field exposure has no effect on cell cycle distribution and apoptosis in U-937 and HCA-2/1cch cells. *Bioelectrochemistry* 2001;53:137-140.
 17. Simkó M, Kriehuber R, Weiss DG, LubenRA. Effects of 50 Hz EMF exposure on micronucleus formation and apoptosis in transformed and nontransformed human cell lines. *Bioelectromagnetics* 1998;19:85-91.
 18. Khaki AA, Zarrintan S, Khaki A, Zahedi A. The effect of electromagnetic field on the microstructure of seminal vesicles in rat: q light and transmission electron microscope study. *Pak J Biol Sci.* 2008;11(5):692-701.
 19. Baharara J, Parivar K, Oryan S, Ashraf A. Effects of low frequency electromagnetic fields on gonads and fertility of female balb/c mouse. *Arak University of Medical Sciences Journal.* 2006; 9(2):1-11.
 20. Roshangar L, Hamdi BA, Khaki AA, Rad JS, Soleimani-Rad S. Effect of low-frequency electromagnetic field exposure on oocyte differentiation and follicular development. *Adv Biomed Res.* 2014;3:76. doi: 10.4103/2277-9175.125874.
 21. Khaki AA, Mohadjel MA, Thubs R. The effects of an EMF on the boundary tissue of the testis of rat. *Folia Morphol.* 2006;65(3):188-194.
 22. Barnes FS. Some engineering models for interactions of electric and magnetic fields with biological systems. *Bioelectromagnetics.* 1992; Suppl 1:67-85.
 23. Levin M. Bioelectromagnetics in morphogenesis. *Bioelectromagnetics.* 2003;24:295-315.
 24. Cheronff N, Rogers JM, Kavet R. A review of the literature on potential reproductive and developpmental toxicity of electric and magnetic fields. *Toxicology.* 1992;74: 91-126.
 25. Ahmed E, Mohd Ali A, Homa D. Long-term exposure of male and female mice to 50 HZ magnetic field: effects on fertility. *Bioelectromagnetics.* 2002;23:168-172.
 26. Huuskonen H, Juutilainen J, Komulainen H. Effects of low frequency magnetic fields on fetal development in rats. *Bioelectromagnetics.* 1993;14: 205-213.
 27. Chiang H, Wu RY, Shao BJ, Fu YD, Yao GD, Lu DJ. Pulsed magnetic field from video display terminals enhances teratogenic effects of cytosine arabinoside in mice. *Bioelectromagnetics.* 1995;16:70-74.
 28. Soleimani Rad J, Rowshangar L, Karimi K. The Effect of Electromagnetic Field on Fallopian Tube. *IFFS 2001 Selected Free Communications, Monduzzi Editore, International Proceedings Division, Melbourne (Australia), 2001; November 25-30.*
 29. Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. *Endocr Rev.* 1996;17(2):121-55.
 30. Williams GT, Smith CA. Molecular regulation of apoptosis: genetic controls on cell death. *Cell.* 1993;74:777-779.
 31. Wyllie AH. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature.* 1980;284:555-556.
 32. Mailhes JB, Yaung D, Marino AA, London SN. Electromagnetic fields enhance chemically induced hyperploids in mammalian oocytes. *Mutagenesis.* 1997;12 (5):347-351.
 33. Klug S, Hetscher M, Giles S, Kohlsmann S, et al. The lack of effects of non thermal RF electromagnetic fields on the development of rat embryos grown in culture. *Life Sci.* 1997; 61:1789-802.
 34. Cao YN, Zhang YL. Effects of exposure to extremely Low frequency electromagnetic fields on reproduction of female mice and development of offspring's. *Zhonghua Lao Dong Wei sheng Zhi Ye Bing ZaZhi.* 2006;24:468 -70
 35. Al-Akhras MA, DarmaniH, Elbetieha A. Influence of 50Hz magnetic field on sex hormones other fertility parameters of adult male rats. *Bioelectromagnetic.* 2006;27(2):127-31.
 36. Hjollund NH, Skotte JH, Kolstad H A, Bonde JP. Extremely low frequency magnetic fields and fertility: a follow up study of couples planning first pregnancies. The Danish first pregnancy planner study team. *Occup*

- Environ Med. 1999; 56(4):253-5.
37. Huuskonen H, Saastamoinen V, Komulainen H, Laitinen J, et al. Effects of low-frequency magnetic fields on implantation in rats. *Reprod Toxicol.* 2001;15(1):49-59.
 38. Murat Bakacaka, Mehmet Sühha Bostancı b, Rukset Attar. The effects of electromagnetic fields on the number of ovarian primordial follicles: an experimental study. *Kaohsiung J Med Sci.* 2015;31(6):287-292.
 39. Roushangar L, Rad JS. Ultrastructural alterations and occurrence of apoptosis in developing follicles exposed to low frequency electromagnetic field in rat ovary. *Pak J Biol Sci.* 2007;10(24):4413-4419.
 40. Khaki A, Farnam A, Rouhani S. Androgenic activity evaluation of ginger rhizome in reducing depression in the forced swimming test of rats exposed to electromagnetic field (EMF). *Int J Womens Health Reprod Sci.* 2013;1(2):56-63
 41. Ghanbari Z. Premature thelarche and precocious puberty in a three-year-old girl with granulosa cell tumor. *Int J Womens Health Reprod Sci.* 2013;1(2):72-75.

Copyright © 2017 The Author(s); This is an open-access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.