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Effect of low-frequency electromagnetic field exposure on oocyte differentiation and follicular development

L. Roshangar, B. A. Hamdi,[1] A. A. Khaki,[1] J Soleimani Rad, and S. Soleimani-Rad[2]
Department of Anatomical Science, Neuroscience Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[1]Department of Clinical Analysis, Pharmacy College, Hawler Medical University, Kurdistan, Iran
[2]Department of Gynecology, Alzahra Hospital, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
Address for correspondence: Prof. Soleimani Rad Jafar, Department of Anatomical Sciences, Faculty of medicine, Tabriz University of Medical Sciences, Tabriz, Iran. E-mail: moc.oohay@jrinamieloS
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ABSTRACT

Background:

The effect of electromagnetic field (EMF) as an environmental factor on different organs including female reproductive system is of critical concern. The aim of the present study is to evaluate the effect of low-frequency (LF)-EMF on oocyte differentiation and follicular development.

Materials and Methods:

The experiment was carried out in animal lab of Faculty of Medicine Tabriz University of Medical Sciences. For this purpose, the BALB/c mice were divided into control and experimental group in animal lab. The pregnant mice in the experimental group were exposed to 3 mT EMF field, 4 h/day during the pregnancy period. The LF-EMF was produced by a system using 50 Hz alternative current, in the control group the pregnant mice were kept in a similar condition without exposure to EMF. The neonatal mice from both groups were sacrificed immediately after birth and their ovary was dissected apart and prepared for light and electron microscopy.

Result:

Microscopy revealed that in the experimental group, in comparison to control group, oocyte nests were mostly broken and irregularly arranged. The primordial follicles were less developed and nuclei of oocytes with an electron microscope appeared heterochromatic, shrunken and had vacuolated cytoplasm.

Conclusion:

It is concluded that exposure to EMF during the developmental period could affect both oocyte differentiation and folliculogenesis and may result in reduced fertility, by decreasing ovarian reservoir.

Keywords: Atresia follicle, electromagnetic field, folliculogenesis, mice, neonatal

INTRODUCTION

During the 20th century, the exposure to the electromagnetic field (EMF) becomes an important source of concern about the possible effects in the living organisms. The artificial sources of electromagnetic radiation have risen tremendously because of the on-going needs on electricity, telecommunications and electronic devices. In this context, World Health Organization established in 1987,[1] the International EMF project in order to assess health and environmental effects of exposure to EMF in the frequency range from 0 to 300 GHz. A number of studies showed that exposure to extremely low-frequency EMF did not induce any adverse effects on spermatogenesis and reproductive capacity in experimental animals and human.[2,3,4,5] In contrast, some studies conducted by other investigators showed clear damage to spermatogenesis.[1,6,7,8,9,10] Therefore, more careful and detailed studies need to be carried out to determine whether EMF exposure can induce adverse effects on spermatogenesis and reproductive capacity. From another point of view, many studies have been carried out or are in progress about the various effects of radiation emissions regarding the behaviors, cancer central nervous system, sleep, children, cardiovascular system, immune function and development.[11]

It has been suggested that electric and magnetic fields at environmental levels may extend the lifetime of free radicals and result in deoxyribonucleic acid (DNA) damage.[12,13] EMFs could have a harmful effect on cellular metabolism by affecting biochemical reactions, behavior of charged molecules and ion channels, synthesis of macromolecules.[13,14] In view of the fact that embryo-fetuses and young, growing animals are more susceptible to the toxicity of xenobiotics, exposure to any xenobiotic during gestational and lactation stages of gonadal development may lead to permanent damage to the gonads. The present study was carried out on mice to investigate the potential adverse effects of neonatal exposure to 50 Hz EMF on the folliculogenesis in adulthood. [15,16]

MATERIALS AND METHODS

Animal husbandry, maintenance and EMF exposure facility

Approximately, 8-9-week-old male and female specific-pathogen-free, BALB/c mice weighting 30 ± 5 g were quarantined and acclimatized for 1 week before the experiment all investigations were conducted in research animal house unit in Department of Histology, Faculty of Medicine, Tabriz University of Medical Science, Tabriz, Iran.

Two female mice were placed into the cage of one male mouse overnight. Vaginal plug was performed the next morning and this was designated as day zero of pregnancy if sperm were detected. A total of 30 pregnant females were then housed in a room maintained at a temperature of 23 ± 1°C, with good ventilation, relative humidity of 50 ± 10% during the exposure period and

artificial lighting from 08:00 am to 08:00 pm.[17,18]

The pregnant mice were divided into two groups, each with 15 pregnant mice. The experimental group was exposed to 3 mT (50 Hz) magnetic field in the EMF producing device for the cage, for 4 h/day. The exposure time was from 8:00 am to 12:00 noon for 21 days while, the other group (control) were kept in the same environmental condition, but without exposure to EMF. After delivery, the half female pups from both groups were sacrificed then remove the ovary. The other half female pups from both groups were kept under normal condition until reach adulthood then, they were sacrificed by cervical dislocation and their ovaries were removed.

Histological examination studies

The ovaries were dissected into small pieces and were immediately fixed in alcoholic Bouins solution for 24 h, then dehydrated and finally embedded in paraffin and were sectioned serially in 5 mm thick sections. The sections were stained with hematoxylin and eosin and studied under light microscope (LM). Morphometric studies were performed in five sections from each ovaries.

Electron microscopy preparation

For transmission EM (TEM) the left ovarian samples obtained from mice in each group were cut into pieces using a scalpel, usually about 1 mm^3.

Primary fixation with 2.5% glutaraldehyde in a 0.1 M phosphate buffer (purchased from Thuringowa Central Qld 4817, Australia) and post-fixed in 1% aqueous osmium tetroxide (TAAB, Berkshrie UK). The pieces were then dehydrated through graded concentration of ethanol and embedded in resin. One micron semi-thin sections were stained with toluidine blue. Ultra-thin sections from selected blokes were stained with uranyl acetate and lead citrate and observed in a LEO 906 type TEM (Oberkochen, Germany).

Statistical analysis

The data were analyzed and compared with the control group using (Student *t*-test). The $P < 0.05$ was considered as significant throughout this study.

RESULTS

Neonatal ovarian effect of EMF exposure

LM studies

LM showed that in 2-day-old neonatal mouse ovary from the control group, oocytes and oocyte

nests were already appeared clearly and surrounded by cuboidal cells. The primary follicles have a large oocyte and a layer of granulosa cells [Figure 1].

In the experimental group, numerous oocyte nests were in the stroma of the ovaries and in some cases each oocyte is partially surrounded by pre-granulosa cells, the oocyte and the oocyte nest appeared to be fewer in comparison to that of the control group. Some oocytes were present as dispersed among stromal cells and few oocyte nests were broken and some primordial follicles were binucleated [Figure 2].

The follicular nests appeared denser than in the control group, it seems that the stromal cells were looser in the experimental group.

TEM studies

Ultra structural studies revealed that developing ovaries in newborn mice showing oocytes surrounded by follicular or pregronulosa cells. Oocyte with huge irregular nucleus and highly dense, nucleolus in the control group [Figure 3].

The electron micrographs of the ovary from EMF exposed rats show that oocyte have irregular nucleus with condensed heterochromatin, oocyte were surrounded by pergranulosa or follicular cells, the cytoplasm showed a higher number of irregular lamellae and the connective tissue was arranged around oocyte like multilayer as lamella with vacuolated cytoplasm. The follicular nest in the experimental group appeared denser than in the control group. The oocyte had numerous vacuoles in the cytoplasm, which were not observed in the control group [Figure 4].

Adulthood ovarian effect of EMF exposure

LM studies

In the control group, healthy oocytes in folliculogenesis showed the granulosa layer in a graffian follicle is enclosed by theca interna, which is rich in cells while the theca externa is rich in collagen fibers.

In the exposed group, the low magnification of cortex contain many follicles in different stages of development but most of them have broken granulosa or thecal layer and their oocyte have lost distinct nuclei ultimately becoming atretic follicle. Figure 5a shows the follicles with intact oocytes contain several layer of granulose cells and fail to form antrum fluid follicles. The alteration of oocyte and the presence of granulosa cells with their separation from neighboring cells constitute the main feature of follicular atresia and also characteristics of apoptosis rupturing of zona pellucida with vacuolization of theca interna area [Figure [Figure5a5a and andb].b]. The percentage of follicles at different stages and also atretic follicles in adult group are presented in Table 1.

TEM studies

Transmission electron micrograph of developing follicle from adult mice ovary shows many granulosa cells separated by basal lamina from the thecal layer. Theca interna is distinguished from theca externa as the latter is surrounded by more fibroblast and connective tissue with many blood vessels appeared between theca cells.

In the experimental group, the ultrastructral studies show that the nuclei of oocytes were shrunken and had lost their regular shape. The irregular granulosa cells contained nuclei with chromatin condensation, several autophagic vacuoles around the nucleus of granulosa cell and separation of the cell from neighboring cells indicated an apoptotic state. Plenty vacuoles were present in the thecal cell and oocytes. Condensation of the chromatin to the margin of the nucleus is evident in contrast with the control group.

DISCUSSION

The present study was carried out in mice to determine the potential adverse effects of neonatal exposure to 50 Hz EMF on folliculogenesis and fertility. The results obtained in the present study were to investigate the effect of EMF exposure during the developmental period on folliculogenesis. Binuclation of primordial follicles with irregular nuclei and broken oocyte. Breakdown of oocyte nests has already been described by.[19,20]

The current study shows that EMF exposure increases degenerative changes and oocyte nest breakdown and follicular formation, undergo a series of incomplete cell division, resulting in clusters called cysts or nest,[21] it has been observed that the cuboidal granulose cells divide more than flat cells.[22,23,24,25]

Findings of the present study also show the nuclei of oocytes were shrunken and had lost their regular shape. In accordance with our findings Cecconi *et al*.,[26] has reported that, most of the nest cells differentiate as nurse cells and contribute to cytoplasm components, including ribosomes to the future oocyte.[20,27]

While the nest-forming cells stops dividing and enter meiosis, becoming oocytes, in the exposed animals the nuclei of oocytes were shrunken and had lost their regular shape the nuclei have multi nucleolus with dark chromatin condensation and had several autophagic vacuoles with different shape and size, with apoptotic bodies.[21,28,29]

It has been reported that EMF exposure has a detrimental effect on the physiological parameters of the majority of exposed follicles and that this detrimental effect on the somatic (pre-granulose) cells also.[30,31] Numerous studies have shown in mammals, the production of mature ova results from a coordinated sequence of events in the ovarian follicle involving various cell types, such as the oocyte and the surrounding granulosa thecal cells. In newborn mouse, oocytes become enclosed in primordial follicle consisting of several enclosed granulosa cells surrounding each oocyte.[16,19,20]

The major goal of this study is that to investigate the effects of EMF-exposure during fetal development on folliculogenesis in adulthood mice. The results indicate loss of oocytes and degenerating of granulosa cells, which comes in accordance to the results found by.[32,33] The appearance of condensed nuclei in granulose cells to become atratic follicle and their separation from neighboring cells constitutes the main feature of follicular atresia with broken zona pellucida and also characteristics of apoptosis. This phenomenon has been previously referred to by many authors[34,35,36] and comes in agreement with our findings. Condensation chromatin as a result of EMF exposure has crucial importance and several many authors as it was referred to previously have shown that condensing nuclei to become atretic follicle. A few numbers of studies have also documented that nuclei of oocyte were shrunken and lost their regular shape. The granulosa cells showed chromatin condensation and had several autophagic vacuoles, corresponding to fat droplets in the control group.

Postnatal follicular atresia has linked to cell death, but the underlying molecular mechanisms are not understood.

Figure 5a shows the follicles with intact oocytes contain several layer of granulose cells and fail to form antrum fluid follicles, our hypothesized that, may be the result related either to an increase in granulose cells death or to a lack of proliferation in granulose cells.

The arrangement of oocytes in the condensation of granulosa nuclei and their separation from each other, indicate a landmark of follicular atresia and it is also characteristics of apoptosis. In addition, even though it is often focused on cell death, atresia is also an active cellular process with resorption of follicle involving macrophage infiltration, phagocytosis and migration of fibroblast from the theca and production of collagen, which are some of the processes observed in wound healing. [36,37]

Our findings well agrees with pro-oxidant effect of EMF and that oxidants are well-known apoptosis-inducing factors.[33,36,39]

According to Peluso *et al*.,[33] granulosa cells in atretic follicles undergo nuclear condensation and cytoplasmic blobbing.

TEM showed these types of changes occurred exposed granulosa cells and appearance of dead cell bodies is characteristic of late apoptosis.

The incidence of the mechanisms underlying follicular atresia are not well-known, DNA damage, which can be initiated by oxidative free radicals, had been proposed as a possible mechanism that leads to the activation of the apoptotic cascade in atretic follicles.[40] In support of this hypothesis, it is shown that exposure to EMF has a pro-oxidant effect.[38]

This is in contrast to a recent report that EMF induced alteration in the oocyte itself, could be considered as a proapoptotic status of oocyte. Irregular morphology of nucleus could be an

indication of changes in nuclear skeleton. Change in cytoskeleton protein and degradation of the nuclear lamina is considered as a trigger of apoptosis cascade.[41]

Previous studies have demonstrated that a significant decrease in granulosa cell proliferation has been related to granulosa cell apoptosis[42,43] an intriguing explanation for the current results may be that EMF is capable of inducing granulosa cell apoptosis. The vacuolization of theca interna shows clearly the thecal cells are oriented differently in these follicles. It appears they have been radiated toward the antrum, whereas in the control group, the increased endothelial cells death observed in the blood vessels of the theca of basal atretic follicles,[44] it can be speculated that the blood flow through these blood vessels is reduced, whether this is a cause or effect of basal lamina layer.

On the other hand, the atresia of follicles leads to loss of the whole follicle and not just death of single cells, even if death of one cell, such as oocyte, is observed early in the process. In addition, even though the focus is often on cell death, atresia is also an active cellular process with resorption of the follicle involving macrophage infiltration, phagocytosis, migration of fibroblasts from the theca and production of collagen, which is some of the processes observed in wound healing.[45,46] Which cell die first and perhaps initiate atresia varies during follicular development? During follicle growth in our result, different cell may vary in their susceptibility to death and if these cells are irreplaceable then this would result in follicular atresia. At the bovine preantral stage, oocytes are reported to be the first to die,[47] in contrast to the antral stage where granulose cells die first and in one form of atresia thecal cells, including endothelial cells,[48,49] also die very early in atresia. In fact, cell death is a normal part of tissue homoeostasis. Therefore, a limited amount of cell death of granulose or thecal cells would not be unexpected in a control group follicle contributing to the difficulty of defining precisely when a follicle has commenced atresia[50,51,52] and estimation of the required level of cell death.

CONCLUSION

The aim of this work has shown that EMF-exposure causes reproductive and developmental toxicity effect of oocytes degeneration in mice. The presets EMF-exposure model can be used to improve the viability of ovarian tissue in order to grow follicles to protect reproductive organ from EMF effect. The potential health effects of EMF should be continually reassessed as new research results become available. EMF exposure reconsidered as new scientific information on radiation and health risks is produced. However, further research should be done in order to clarify many unknown aspects of the impact of EMF on human reproduction.

Footnotes

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Conflict of Interest: None declared.

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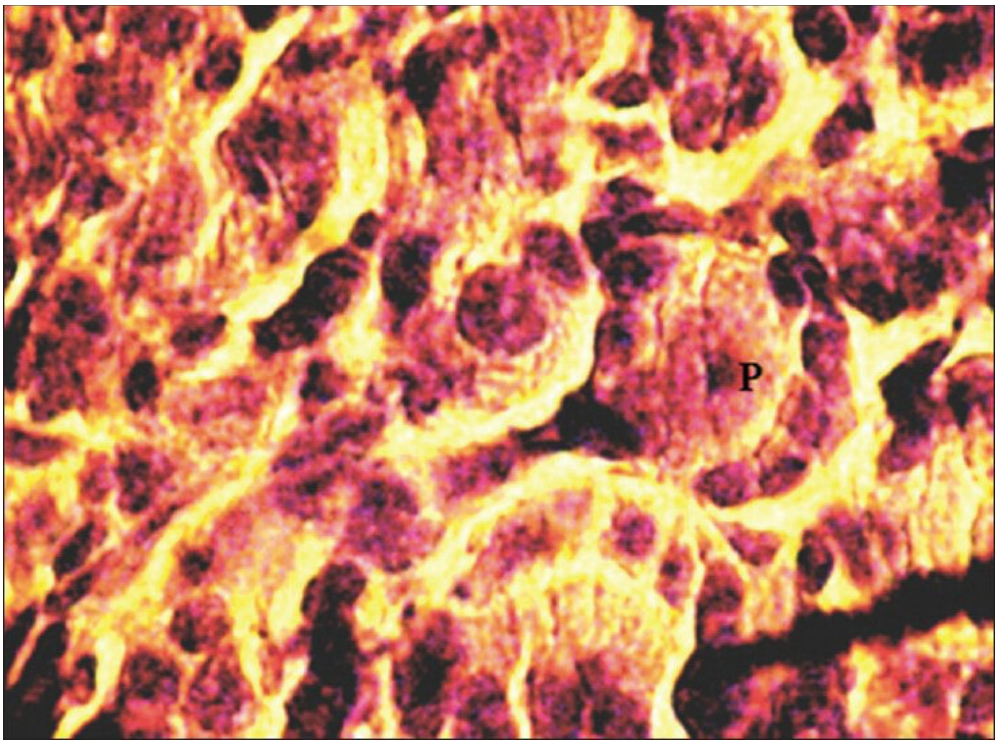
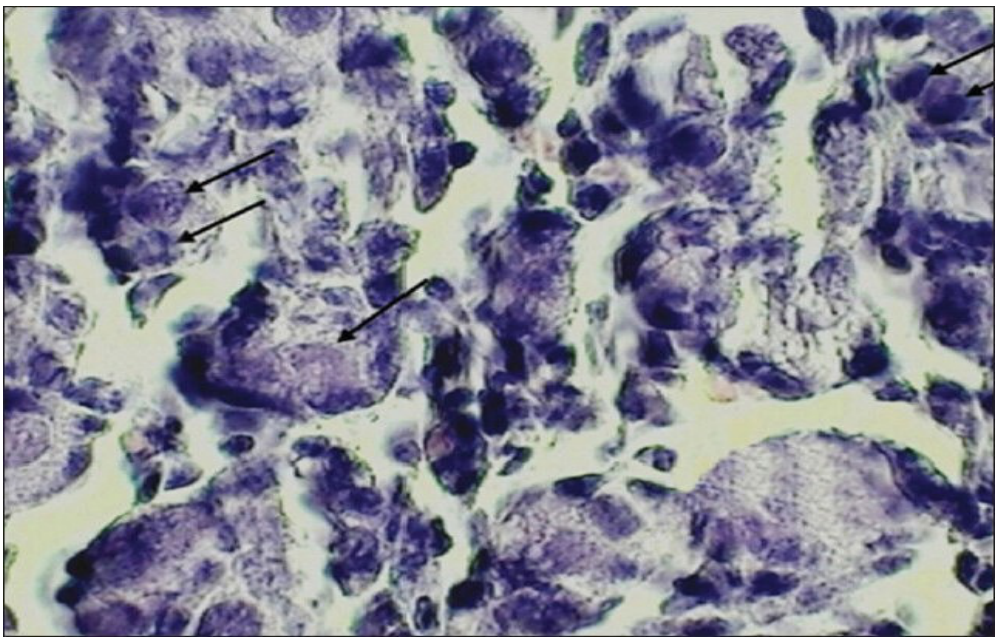
Figures and Tables

Figure 1



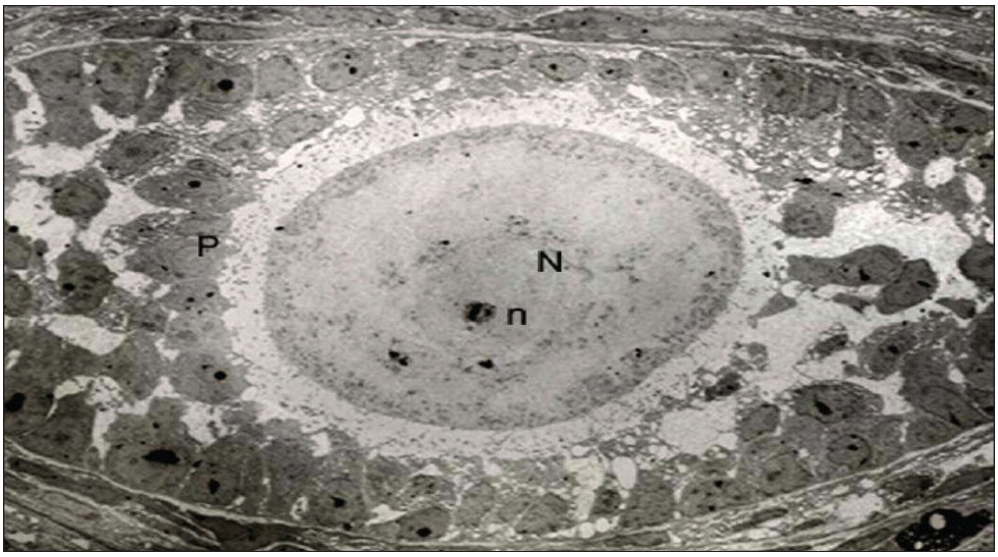
Light microscopic micrograph from ovary of 2-day-old neonatal mouse control group. Note differentiating oocyte nest, primordial follicle, follicular nests and oocyte clusters (H and E, ×780)

Figure 2



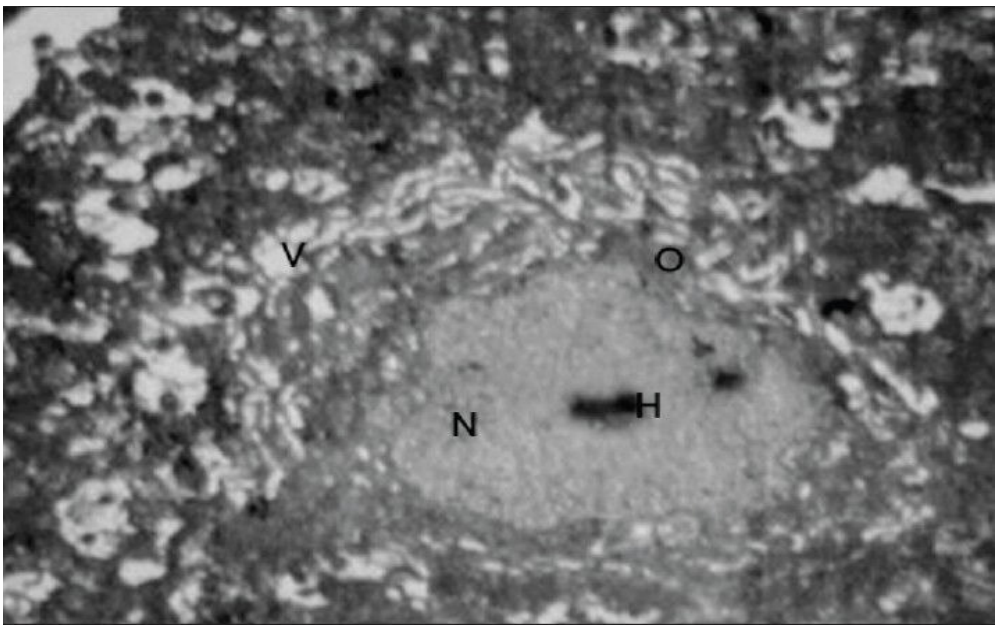
Light microscopic micrograph from neonatal female mouse exposed to electromagnetic field. Note scattered oocyte (arrow), binucleated oocytes (double arrow) and rapture in arrangement of pregranulosa cell (H and E, ×780)

Figure 3



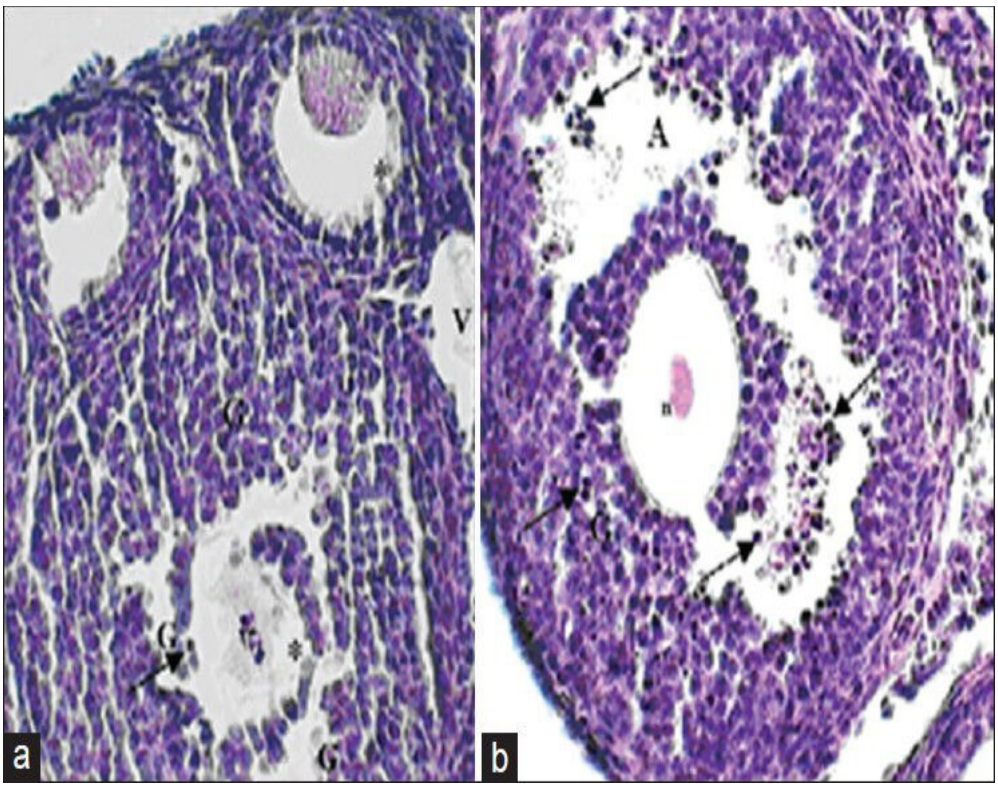
An electron micrograph of developing follicle from a control neonatal mouse ovary. Note oocyte, nucleous (n), nucleolus (n), surrounded by pre-granulosa cell (g), ×2704

Figure 4



An electron micrograph from exposed neonatal female mouse to (electromagnetic field). Note oocyte (o), irregular nucleus membrane with shrunken nuclei (n), multinuclouses with deferent size of heterochromatin (n), vacuolated cytoplasm (v), ×2704

Figure 5



Light microscopic micrograph from exposed adulthood ovaries to electromagnetic field. (a) Rupturing and thinning in zona pellucid (*) and separation granulosa layer (g), vacuolization (v), rupturing of nuclei of the oocyte (n) H and E, ×780. (b) Antral atresia of follicles, location of gronulosa cells (g) and examples of dying cells (arrow heads) lead in the antrum follicle fluid and

irregular shape of nucleous (n) H and E, ×780

Table 1

Follicular phase	Experimental %	Control %
Primordial follicle	24.18	35.76
Primary follicle	10.21	11.05
Growing primary follicle	12.56	14.11
Secondary follicle	15.23	16.12
Antral follicle	10.08	13.47
Atretic follicles	27.84	9.49

Percentage of different follicles in control and experimental groups in adult ovaries

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