

## The Effect of Non-ionizing Electromagnetic Field on Oocyte Apoptosis in Rats by Tunel Method

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### ABSTRACT

In recent year's attention to safety effects, environmental and Society health, extremely low frequency electromagnetic fields (ELF- EMF) and radio frequency electromagnetic fields (RF-EMF) increased. "Teratogenic", the name is given to the development of the embryo or fetus damaging factors during pregnancy. Overall, teratogens include environmental factors and factors related to the mother.

**Materials and Methods:** 30 rats were randomly taken from laboratory animals and at first age and weight were determined. For testing, 3 months rats were selected and randomly divided into three groups. The control group consisted of 10 rats without any treatments were kept in normal conditions. The second group and the third group of mice for 8 weeks (three weeks intrauterine and five weeks ectopic) and for 13 weeks (three weeks intrauterine and ten weeks ectopic) were influenced by 50 Hz of a magnetic field respectively. To investigate the apoptosis of samples that taken from three groups, they were fixed in 10% buffered formaldehyde. After that samples were embedding in paraffin and finally by immunohistochemistry tunnel be painted. Finally, by light microscopy were studied.

**Results:** EMF radiations increase the harmful effects on the formation of ovarian follicle and ovule implantation. Studies on the effects of electromagnetic fields on ovarian follicles have shown that oocyte nucleus becomes smaller and change shape. Apoptotic cells in the groups affected by electromagnetic waves compared to the control group had significant changes.

**Discussion:** Exposure to electromagnetic fields during embryonic development can cause morphological changes in the oocyte and to affect differentiation of oocyte and folliculogenesis and resulting in decreased ovarian reserve leading to infertility or reduced fertility.

**Keywords:** Electromagnetic field, Follicle, Apoptosis

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### 1. Introduction

Human progress in the field of new technologies in the field of health has implications that require management and some health interventions. Recently, interest in electromagnetic fields due to increasing concerns about the potential effects of EMF, both on human health and animals has increased (1,2). We live in a world that is completely surrounded by earth's geomagnetic field with 0/50e and electromagnetic pollution is caused by man-made sources. Damaging effects of electromagnetic fields Depends on the frequency (wavelength), the density of field and the duration of radiation(1). Extremely low frequency electromagnetic fields (ELF- EMF)...non-ionizing radiation that has a frequency range of 1 to 100 Hz and use from a variety of sources such as power lines, electric transport systems, and home appliances with frequency of 50 or 60 Hz that emit waves with long wavelength (3). Most electrical appliances work in the frequency range between 50 and 60 Hz and further studies of the impact of electromagnetic radiation on living organisms is done in this frequency range. 50 Hz is corresponding to the wavelength of 3,500 kilometers, which is close to the Earth's radius (3). Many studies have been carried out on the possible effects of low frequency fields on human health (4,5,6). It seems that electromagnetic fields can cause infertility in women (7,8). ELF-EMF has

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devastating effects on the reproductive system of female rats with reducing the number of FLUSHED blastocysts and increasing the height of epithelial cells of the fallopian tube (9). ELF-EMF radiation can cause changes in the uterus and ovaries in rats (10), to influence follicular development and estrus cycle and cause to birth defects, miscarriage and premature birth (11).

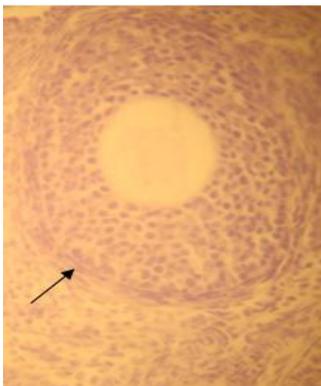
Development of tissues, organs and systems during embryogenesis is sensitive to toxic agents (10). EM fields potentially affect fetal development, but their mechanisms still not fully understood (12). Based on the studies, parents who are exposed to electromagnetic fields (PARANTAL EXPOSURE) with frequencies from 50 to 60Hz both before and during pregnancy can have an important role in the development of childhood cancer (13). It also has been shown that exposure to extremely low frequency electromagnetic fields during pregnancy can cause adverse effects in pregnant female rats and the development of their babies. Abortion, fetal loss, malformation and growth delay in children (OFFSPRING) of pregnant rats affected by EMF (14). Pregnant physiotherapists during pregnancy because of their jobs are affected by microwave radiation have an increased risk of miscarriage. Living in proximity (closer than 50 meters) of ELF-EMF source during pregnancy can cause suboptimal uterus growth (15). Another study also found that exposure of pregnant women between the first to 20th days of pregnancy reduces the number of viable fetuses per mother (16). However; other studies have given conflicting results. Pregnant mice exposed to a magnetic field with a frequency of 60 Hz in the 6th to 20th day of pregnancy significant biological effect was not observed in the fetus and puppies (17). In another study it was shown that the electromagnetic field radiation at a frequency of 60 Hz in the 6th to 21th was not have biological effect on the mother's pregnancy and the first and second generation (18). In a study on pregnant wistar rats were exposed to low frequency electromagnetic field has not been reported any changes in body weight and the number of embryos (18).

Due to contradictory results reported, the aim of this study is to evaluating effect of electromagnetic fields exposure during embryonic development, morphological changes in the oocyte and to affect differentiation of oocyte and folliculogenesis, resulting in decreased ovarian reserve leading to infertility or reduced fertility.

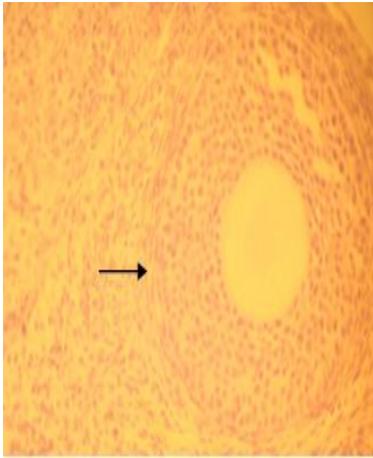
## 2. Materials and Methods

30 rats were taken randomly from laboratory animals and at first, age and weight were determined. 3 months old rats selected for testing, then animals were randomly divided into three groups: Control group N=10, T1=10 and T2=10. During the experiment, three groups will be maintenance and feeding in the same condition. Experimental groups affected by the 50 to 60 Hz magnetic field. Namely, first group for 8 weeks (3 weeks intrauterine + 5 weeks ectopic), the second group for 13 weeks (3 weeks intrauterine + 10 weeks ectopic). After above periods, the animals anesthetized with Pentobarbital (40mg) then remove the ovaries and isolated oocytes, for evaluations of apoptosis in the oocytes cell by light microscopic, this cell were immediately fixed in formaldehyde 10% and them embedding in paraffin and finally by TUNEL immunohistochemistry will be stained and studied cells by light microscope.

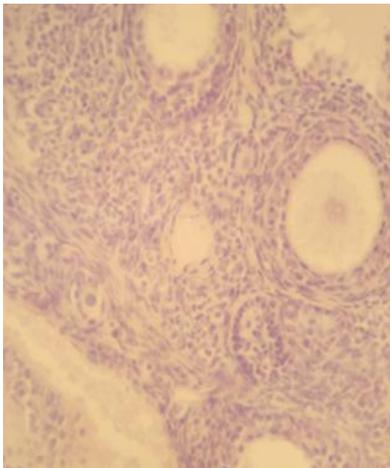
## 3. Results



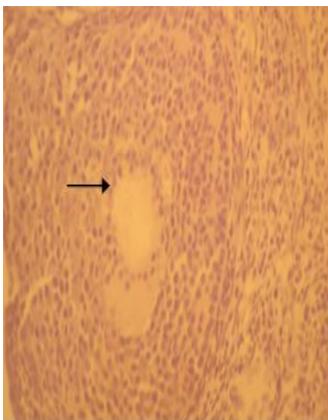
**Figure 1:** micrograph of Light Microscope from control group, growing primary follicles in the bed of ovarian stroma clearly seen. Granulosa cells around the growing follicles with theca layers are regularly can be seen. Zona pellucida with specified thickness in the follicles is evident. X40 Hematoxylin-eosin stains (H & E)



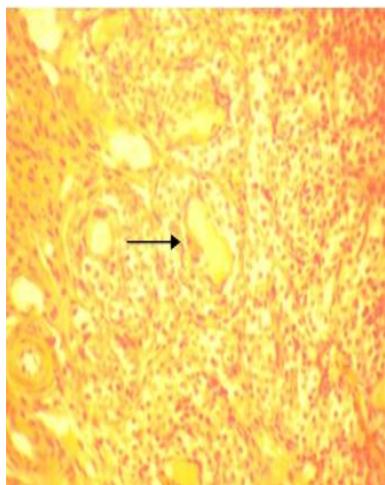
**Figure 2:** micrograph of Light Microscope from control group two, growing primary follicles with identified granulosa cells which has core of euchromatin. Zona pellucida layer (Z) around the oocyte has acidophilus color and homogenized and clear. Theca layer (T) on a regular basis is visible around the follicle. X40 Hematoxylin-eosin stains (H & E)



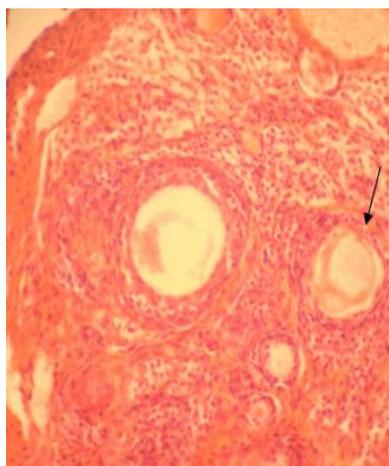
**Figure 3:** micrograph of Light Microscope from the experimental group 1-1, growing ovarian follicles with immature layers of granulosa cells, relatively non-visible Zona pellucida layer with discrete theca layer in stromal bed has seen as it is granular. X40 Hematoxylin-eosin stains (H & E)



**Figure 4:** micrograph of Light Microscope from Experimental group 1-2, growing primary follicles with relatively irregular arrangement with discrete theca layer and uneven thickness of zona pellucida layer in some parts of the follicles is visible. X40 Hematoxylin-eosin stains (H & E)



**Figure 5:** micrograph of Light Microscope from Experimental group 2-1, the construction of ovarian stroma torn apart and dilated blood vessels and blood congestion is evident. Also, infiltration of blood leukocytes is visible. X40 Hematoxylin-eosin stains (H & E)



**Figure 6:** micrograph of Light Microscope from Experimental group 2-2, disappearance and destruction of granulosa of the growing primary follicles were evident. Irregular oocyte in follicle is quite evident (Arrows). Also, the structure of ovarian stroma is disrupted. The oocyte cytoplasm like acidophilus plates within the follicles is visible. Also, accumulation of collagen fibers in the form of fibrosis is seen in part of the ovarian stroma. X40 Hematoxylin-eosin stains (H & E)

**Table 1:** The oocyte apoptotic cells in different groups affected by EMF

Groups	Group 1	Group 2	Group 3
The apoptotic cells in terms %	5.1 ± 0.41	12 ± 0.41	15 ± 0.41

#### 4. Discussion

Study the biological effects of electromagnetic fields is considered due to the increasing use of devices generating electromagnetic waves and its possible effects on reproduction, fertility and embryonic development(19,24). This study aimed to investigate the effects of electromagnetic field with a frequency of 50 Hz on folliculogenesis and differentiated oocyte of newborn baby during pregnancy that has been affected by the electromagnetic field.

Mouse ovaries during the post-natal period are rich in quiescent and early-growing oocyte that is surrounded by a layer of granulosa cells sex (20,26). EMF exposure during embryonic development can affect folliculogenesis (6) and cause adverse effects on fertility in female mammals (22). The study found that the electromagnetic field can increases the harmful effects on the formation of follicles and ovule implantation.

Shrink of oocyte nucleoli and the irregular shape of the oocytes were observed in this study. Maturation, fertilization and embryonic development before implantation (pre-implantation embryo development) depend on oocyte growth and differentiate follicular cells around it (21). Irregular morphology of the core can be a sign of changes in nuclear structure (23). In a study conducted by Bakacak M on electromagnetic field effects on ovarian follicles have shown that oocyte nucleus becomes smaller and change shape (15). Figure 6 shows the zona pellucida in follicles are highly degraded and are not visible. Also, WBC aggregations for local or topical infiltration are seen around the follicles. Based on the results, exposure to extremely low frequency magnetic fields may impair fertility of female mammals by reducing the ability of follicles to reach the stage of development which is an essential prerequisite for successful reproductive .

Believed to be the development of EMF exposure in daily life in the future by reducing the supply of egg cell in the ovaries leads to an increase in infertility (7,25,26). It has been shown that Follicle atresia in the ovaries is associated with apoptosis of granulosa cells .Programmed death (apoptosis) of ovaries' granulosa cells in the presence of electromagnetic fields has been reported (23). Also,leukocytes aggregations around the follicles are seen to from local infiltration destruction of granulosa cells is under the influence of the electromagnetic field in growing primary cells. EMF exposure during embryonic development affects the process of ovulation, folliculogenesis, loss of egg cells, degeneration of granulosa cell and decreasing ovarian reserve that finally resulting in infertility.

## 5. Conclusion

According to the findings of this study, it seems that exposure to electromagnetic fields during embryonic development can affected morphological changes in the oocyte, differentiation of the oocyte and folliculogenesis, resulting in decreased ovarian reserve leading to infertility or reduced fertility.

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