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## CAN HISTOLOGICAL AND HISTOMORPHOMETRICAL CHANGES BE INDUCED IN RAT MANDIBULAR CONDYLE FOLLOWING OVARIECTOMY AND LONG-TERM EXTREMELY LOW FREQUENCY MAGNETIC FIELD EXPOSURE?

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### ABSTRACT

*The quantity and quality of maxillary and mandibular bone have long been a focus of attention for dental clinicians. The mandibular condyle is a major growth site and plays an important role during mandibular growth. The aim of the present study was to evaluate the effects of long-term extremely low frequency magnetic field (ELF-MF) and hormonal changes produced by bilateral ovariectomy on the histologic and histomorphometric structure of rat mandibular condyle.*

*Forty mature female Sprague-Dawley rats were randomly divided into four different groups (n =10): control (Cnt), ovariectomy (OVX), ELF-MF exposure (ELF-MF), ELF-MF exposure with OVX application (ELF-MF+OVX). All rats were subjected to bilateral ovariectomy except those in Cnt and ELF-MF groups. ELF-MF and ELF-MF+OVX groups were exposed to 1.5 mT ELF-MF during 6 months, 4 h a day. After all applications, left condyle of rats were removed to examine histopathologically. Some histopathologic changes were observed, such as irregular appearance in bone marrow cavities in rat condyles of OVX, ELF-MF and ELF-MF+OVX groups. Some disorders in transition from hypertrophic field to ossification layer and irregular appearance in calcification were determined in OVX group. In OVX+ELF-MF and ELF-MF groups, there was significant disruption and latency in calcification and ossification areas. However, no significant differences were found in the thickness of the condylar cartilage layer between groups ( $P > 0.05$ ). It was suggested that long-term ELF-MF exposure and ELF-MF exposure with ovariectomy application can affect the histologic structure of rats' condyle.*

*Consequently, it was concluded that long-term ELF-MF exposure and ovariectomy application can induce histopathological changes in the structure of the condyle and ELF-MF exposure cannot alter ovariectomy-induced changes.*

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**Keywords:** ELF magnetic field, ovariectomy, mandibular condyle, rats.

### Introduction

The most important function of the skeleton is to give structural support. This function is related to the adaptive capacity of osseous tissue: a change in functional demands stimulates deposition of bone (34). The growth of the skull is governed and affected by a number of factors. The morphology of the temporomandibular joint reflects the complex development of the entire skull (14). A number of normal and abnormal processes can cause changes of the maxillary and mandibular bone tissue (6). A growing body of epidemiological, clinical, and experimental evidence suggests that ovarian steroids, particularly oestrogen, have the potential to modify the metabolic activity of joint tissues (8). Prevention of bone loss due to aging, menopause (hormonal changes), immobilization, or harmful external stimulants (magnetic fields) requires an understanding of the factors that normally regulate remodelling activity. Physical stimuli and, in particular, mechanical loading are most important determinants of bone mass and architecture (34).

The effect of postmenopausal osteoporosis on the mandible is also attracting the attention of dental specialists in recent years. It has been reported that in ovariectomized rats bone loss may occur in the mandibular body and the mandibular condyle (12, 43, 44, 45). A positive correlation has been found between osteoporosis and some jaw conditions, such as low mineral density of the mandible (31, 35), severe residual ridge resorption (21, 48), and excessive tooth loss (5, 35, 47). Some clinical trials have suggested a strong link between tooth loss and estrogen deficiency based on observations that postmenopausal women on estrogen supplement therapy suffered from less severe tooth loss as compared to those who did not receive estrogen (18, 30, 37). The precise relationship between osteoporosis, periodontal mandibular bone, and tooth loss is currently not well understood (7, 11, 20, 29, 32, 49). Despite the growing evidence that ovariectomy (OVX) could be associated with tooth loss, rather conflicting results have been observed in different animal studies on the effect of OVX on the mandibular bone (22, 23, 36, 45).

The studies regarding the biological effects of magnetic fields have intensified with the increasing use of electronic technology, high voltage power transmission, and magnetic resonance imaging in modern society (3, 13, 15, 17, 25, 33,

52). As pointed out by Akdag et al. (1), some of these studies reported that magnetic fields can affect on the behaviors of osteoblast-like cells, stimulate the osteoblasts in the early stages of culture, accelerate cellular proliferation of osteoblasts, and stimulate the increasing of total bone mineral content (4, 9, 10, 24). Despite the increasingly accumulating knowledge on the biological effects of magnetic fields on osteoporosis both in human and experimental animals and the data indicating an association between osteoporosis and tooth loss, no information was found about the histologic and histomorphometric changes that may take place in the condyle of ovariectomized rats exposed to extremely low frequency magnetic field (ELF-MF). Risk of long-term ELF-MF and radiofrequency (RF) radiation exposure is a central issue in the study of possible ELF-MF and RF adverse health outcomes (2, 26). Therefore, long-term ELF-MF exposure was used in this study.

The aim of the present report was to determine whether any changes occur in the histologic and histomorphometric structure of the mandibular condyle, when rats are exposed to ELF-MF for a long time and ovariectomized.

## Materials and Methods

### Animal care and experimental procedure

The experiments were performed on 40 female Sprague-Dawley rats with initial weights of 157-226 g obtained from the Medical Science Application and Research Center of Dicle University, aged 4 months at the beginning of the study. All rats were allowed free access to water and standard pelleted food diet (TAVAS Inc. Adana, Turkey) during the experimental period. The rats were divided into 4 groups of ten animals each: Control (Cnt), Ovariectomy (OVX), ELF-MF Exposure (ELF-MF), ELF-MF exposure with OVX application (ELF-MF+OVX). All rats were subjected to bilateral ovariectomy except those in Cnt and ELF-MF groups four days before the beginning of the experiments under ketamine anesthesia (50 mg/kg, intramuscularly). ELF-MF, and ELF-MF+OVX animals were subjected to 1.5 mT ELF-MF exposure over a 6-month period, 4 h a day, starting at the fifth day after the surgery. The animals were kept in 14/10 h light/dark environment at a constant temperature of  $22 \pm 3$  °C,  $45 \pm 10\%$  humidity. This protocol was approved by the local ethics committee. All animals were handled in accordance with the Principles of Laboratory Animal Care and the rules of Scientific and Ethics Committee of Dicle University Health Research Center.

### Magnetic field generation and exposure of rats to magnetic field

The MF was generated in a device designed by us that had 2 pair of Helmholtz coils of 70 cm in diameter in a Faraday cage (130 × 65 × 80 cm) that earthed shielding against the electric component. This magnet was constructed by winding 125 turns of insulated soft copper wire with a diameter of 1.5 mm. The coils were placed vertically facing one another. The distance between the coils was 47 cm. An AC current produced by an AC power supply (Adakom, Turkey) was passed through the device. The rats were free to move in the methacrylate cage inside the coils. The MF intensities were measured once per

week as 1.5 mT in 15 different points of the methacrylate cage with a Bell 7030 Gauss/Teslameter (F.W. Bell, Inc., Orlando, FL) to ensure homogeneity of the field during the course of the experiment by a person who is not involved in the animal experiment. All field measurements were performed by persons not involved in the animal experiments. Observers were not aware of which group of rats was ELF-MF- or sham-exposed, i.e. the whole study was done blind. No temperature differences were observed between exposure and sham cages during the exposure. ELF-MF, and ELF-MF + OVX animals were exposed to 1.5 mT ELF-MF over a 6-month period, 4 h a day, in methacrylate boxes (43 × 42 × 15 cm). The OVX group were treated like the ELF-MF + OVX group but without ELF-MF exposure in methacrylate boxes. In the control group nothing was applied to rats and they completed their life cycle in the cage during the study period.

On the last day of the study, immediately after the last exposure, the rats were euthanized with a lethal dose of ketamine (100 mg/kg) administered intramuscularly. After euthanization the left condyle was collected, fixed in 10% neutral formalin and decalcified in 5% formic acid.

### Histopathologic evaluation

After decalcification, mandibular condyles were embedded in paraffin and 5 µm thick sagittal sections were stained with haematoxylin and eosin (HE), and tripple stain. Histologic evaluations were made using an ocular scale adapted to a Nikon-Eclipse 400 light microscope under 10× objective magnification. Measurements of the thickness of the condylar cartilage layer were done with the ocular micrometer of the microscope. Measurements were done at the middle of the condyle. In each joint region, three layers were scored: fibrous (articular), proliferative (chondrogenic), and maturative/hypertrophic (cartilaginous) layers. The layers were measured and investigated histologically.

### Statistical analysis

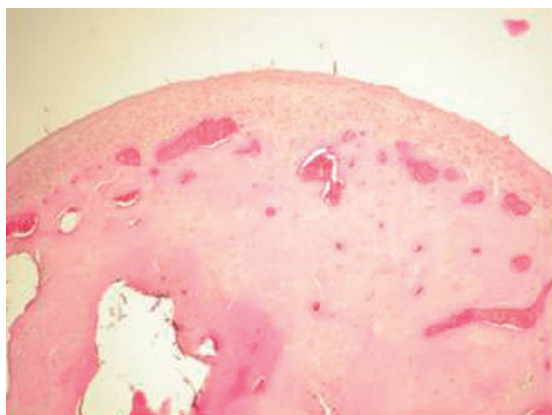
Statistical analysis was performed using SPSS 15 software (SPSS Inc., Chicago, IL, USA). Data were analyzed by Kruskal-Wallis one-way analysis of variance (ANOVA) to determine the differences between the groups in relation to the thickness of the layers in the mandibular condyle. All hypothesis tests used a criterion level of  $P = 0.05$ .

## Results and Discussion

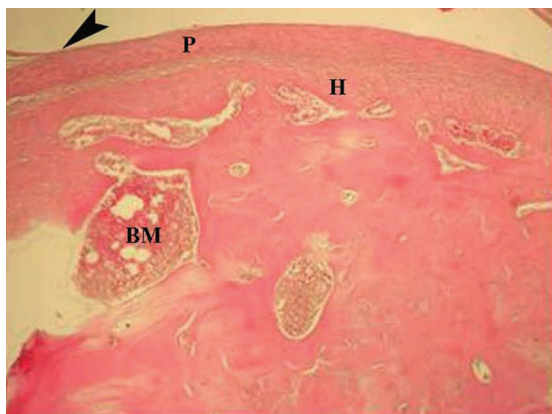
The histopathologic examination of condyles revealed a regular fibrous layer and a clear fibroblastic activity in the fibrous area of all groups except the OVX+ELF-MF group. However, the fibrous layer of the ELF-MF+OVX group was not regular (**Fig. 1**). The proliferative layer of the control group was regular and clear (**Fig. 2**). There was a regular transition from the proliferative layer to the hypertrophic layer in the control group (**Fig. 2**), whereas this layer was thick in the ELF-MF+OVX and OVX groups (**Fig. 1** and **Fig. 3**). It was determined that the cell diameter of the hypertrophic layer increased significantly in the control group. However, an inhomogeneous appearance and decreasing in thickness of the layer was observed in the hypertrophic layer of the



OVX, OVX+ELF-MF and ELF-MF groups. The bone marrow cavities and bone marrow were normal in appearance in the control group. However, irregular appearance of the bone marrow cavities was observed in the OVX, OVX+ELF-MF and ELF-MF groups. While loss in bone marrow was determined in the OVX and OVX+ELF-MF groups, there was an increased amount of bone marrow in the ELF-MF group (Fig. 4). A regular transition from the hypertrophic area to the ossification layer was observed in the control group. However, some disorders in the transition from the hypertrophic field to the ossification layer and irregular appearance in calcification were determined in the OVX group (Fig. 3). In OVX+ELF-MF and ELF-MF groups, significant disruption and latency in the calcification and ossification areas was observed. After histomorphometrical analyses, no significant differences were found between the groups in terms of thickness of the fibrous, proliferative and hypertrophic layers in the condyle of rats (Fig. 5, Fig. 6 and Fig. 7).



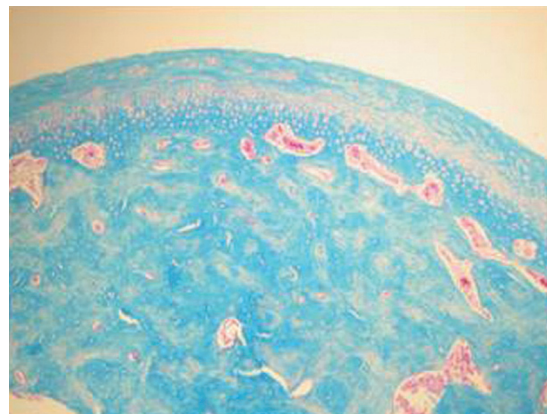
**Fig. 1.** Sagittal section of mandibular condyle of ovariectomy and magnetic field group (ELF-MF+OVX) animals (HE, 100× original magnification).



**Fig. 2.** Sagittal section of mandibular condyle of control group (Cnt) animals: fibrous layer (arrow head), proliferative layer (P), hypertrophic layer (H), bone marrow (BM), (HE, 100× original magnification).

A lot of women worldwide are affected by postmenopausal osteoporosis and side effects of osteoporosis such as periodontal bone loss and tooth loss. Although a large number of authors have undertaken studies to explain the effect of osteoporosis and to solve problems after postmenopausal osteoporosis, some issues still remain unresolved, e.g. regarding the exact

mechanisms of tooth loss after osteoporosis, the effects of osteoporosis on mandibular condyle and the effects of ELF-MF on the condyle in normal and OVX rats. In the present study, we attempted to determine the interaction of ELF-MF with rat mandibular condyle, the effects of OVX on mandibular condyle and whether ELF-MF can affect the histologic and histomorphometric structure of condyle in OVX rats.

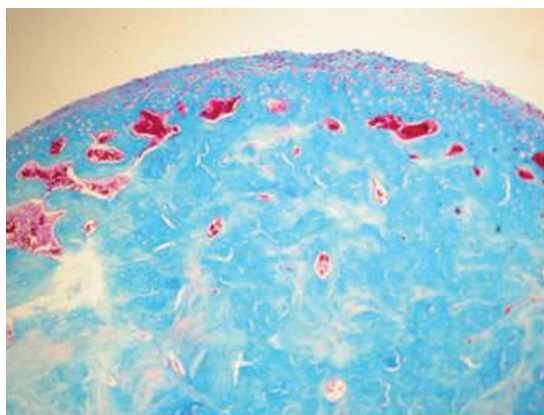


**Fig. 3.** Sagittal section of mandibular condyle of ovariectomy group (OVX) animals (Triple stain, 100× original magnification).

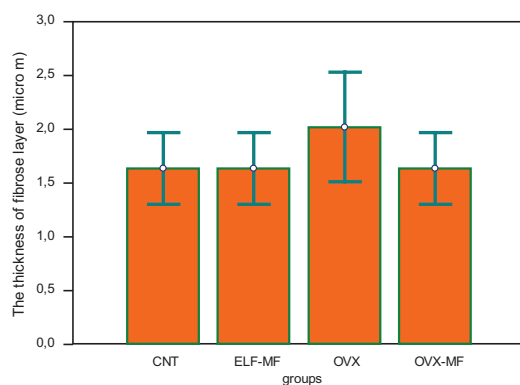
Based on the observed histopathologic changes such as: increased thickness of the proliferative layer, hypertrophic layer contraction, inhomogeneous appearance of cells, irregular appearance of bone marrow cavities, irregularities in transition from hypertrophic areas to ossification and irregular appearance in calcification, it could be suggested that hormonal and physiologic changes in ovariectomized rats can affect the bone structure of mandibular condyle. In the present study, the ovariectomy induced histopathologic changes were consistent with the changes observed in the condyle tissues of ovariectomized rats. Hidaka et al. (19) concluded that the ovariectomy induced changes in the periodontal tissues and alveolar bones of the mandible of rats even in a short time period. Tanaka et al. (43) concluded that net bone loss occurred in the posterior region of the mandibular condyle in ovariectomized rats, owing to region specificity and estrogen deficiency. They suggested that estrogen deficiency and mechanical stress are closely related to structural changes in the mandible, as reported in underloaded long bones. Yamashiro and Takano-Yamamoto (50) showed that ovariectomy significantly increased the total thickness of the condylar cartilage and distal femoral growth-plate cartilage in young growing rats. Pereira et al. (38) obtained that ovariectomy delayed alveolar wound healing after molar extractions in rats. Tanaka et al. (46) found that the OVX rat mandibular condyles dynamically altered their structures under the effects of estrogen deficiency and occlusal loads.

When we compared the OVX+ELF-MF with the OVX group in relation to histopathologic appearance, no significant differences were observed between the groups. However, it could be pointed out that ELF-MF can increase the pathologic appearance with visible changes such as significant disruptions in ossification and calcification, bone marrow loss in the OVX+ELF-MF group. The observations for the OVX+ELF-MF group were not in harmony with some findings of

previous studies. The main cause of this conflict may be due to differences in the magnetic field intensity, duration or shape (pulsed or continuous) and the examined bone. Mavropoulos et al. (32) determined that ovariectomy can induce different changes on different bones of the skeletal system. In the ELF-MF group, according to the observed changes such as delay in ossification and calcification areas, it can be suggested that the ELF magnetic field used in the present study, can affect bone structure of the mandibular condyle. Earlier studies on the health influences of magnetic fields have not ensured clear answers with respect to a possible biological reaction (51). Some authors, however, have become increasingly persuaded that electrical and electromagnetic fields have effects on improvement of bones, tissue repair and other body regions (39, 40, 41). A few studies have shown positive effects of electromagnetic field on bone fractures, osteoporosis, and pseudoarthrosis (42).



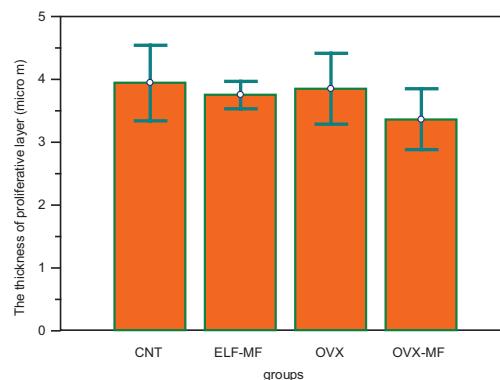
**Fig. 4.** Sagittal section of mandibular condyle of magnetic field group (ELF-MF) animals (Triple stain, 100× original magnification).



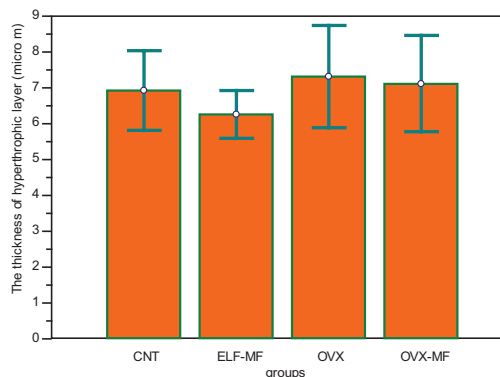
**Fig. 5.** Thickness of the fibrous layer (μm) of mandibular condyle. CNT: Control, OVX: Ovariectomy, ELF-MF: ELF-MF Exposure, MF+OVX: ELF-MF exposure with OVX application. No significant differences were found between groups (Kruskal-Wallis one-way ANOVA). Values are expressed as mean ± S.D. (n = 10).

We showed that ovariectomy and magnetic field exposure for 6 months affected the condylar regions histologically in the experimental group, but the condylar layer thickness did not show significant difference from the control group. In the experimental group of rats the cellular elements and calcification were significantly different from those in the

animals from the other groups. The results of the present study showed that oestrogen deficiency, produced by means of ovariectomy, and magnetic field application in rats, provoked significant changes in the structural properties of the condylar layers. These results also indicate that oestrogen may alter condylar remodelling, leading to degenerative changes in the temporomandibular joint. Similar conclusions were drawn by Fujita et al. (16). In the longer experimental process the results may be quite different. To our knowledge, there are only a few recent similar publication dedicated to the effect of ovariectomy and magnetic field (27, 28) but these factors have not been examined on the temporomandibular condyle.



**Fig. 6.** Thickness of the proliferative layer (μm) of mandibular condyle of mature female Sprague-Dawley rats. CNT: Control, OVX: Ovariectomy, ELF-MF: ELF-MF Exposure, MF+OVX: ELF-MF exposure with OVX application. No significant differences were found between groups (Kruskal-Wallis one-way ANOVA). Values are expressed as mean ± S.D. (n = 10).



**Fig. 7.** Thickness of the hypertrophic layer (μm) of mandibular condyle of mature female Sprague-Dawley rats. CNT: Control, OVX: Ovariectomy, ELF-MF: ELF-MF Exposure, MF+OVX: ELF-MF exposure with OVX application. No significant differences were found between groups (Kruskal-Wallis one-way ANOVA). Values are expressed as mean ± S.D. (n = 10).

## Conclusions

It was concluded that long-term ELF-MF exposure and ovariectomy application can induce histopathological changes in the structure of the condyle and ELF-MF exposure cannot alter the ovariectomy-induced changes. However, further studies are needed in order to explore the interaction mechanisms between ELF-MF and the cells of the mandibular condyle.

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