

Morphological changes in the vertebrae and central canal of rat pups born after exposure to the electromagnetic field of pregnant rats

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ABSTRACT

Several studies have investigated the effects of the electromagnetic field (EMF) on the central nervous system. However, we encountered no studies of the effects of EMF applied in the prenatal period on the offspring vertebrae. The aim of this study is to investigate the effect of a 900 megahertz (MHz) EMF applied to rat dams in the prenatal period on the vertebrae of rat pups. Female Sprague Dawley rats weighing 180–250 g were used in the experiment. Rats identified as pregnant were divided into two groups, control ($n = 3$) and EMF ($n = 3$). No EMF was applied to the control group pregnant rats. EMF was applied to the EMF group rats for 1 h daily on an equal and standard basis on prenatal days 13–21. All newborn rat pups were divided into pup control ($n = 6$) (PC) and pup EMF ($n = 6$) (PEMF) groups, and no treatment was performed on either. All animals were decapitated on day 32, and the spinal cord in the upper thoracic region was harvested. Vertebral tissues were subjected to routine histological procedures. Histopathological examination revealed that PEMF group vertebral cartilage had been converted into bone tissue. Comparison of central canal diameter and area values between the PEMF group and the PC group revealed statistically significant increases in the PEMF group ($p = 0.000$ and $p = 0.001$, respectively). Statistical analysis revealed no significant difference in mean body weights between the two groups ($p > 0.530$). Based on these findings, we think that 900 MHz EMF applied in the prenatal period affects the development of the vertebrae. This effect causes pathological changes in the rat pup vertebrae. These findings now raise the question of whether EMF also has an impact on neurological and neurosurgical diseases involving the vertebrae.

1. Introduction

Electromagnetic field (EMF) exposure has increased rapidly in recent years. Dependence on technological devices is a particular factor in this increased exposure (Keleş et al., 2019b). Although many studies have been performed on this subject, no definite conclusions have been reached (Baş et al., 2013; Odaci et al. (2008); Li et al., 2019). Some studies suggest that EMF is harmful (İkinci et al., 2013; Şahin et al., 2015; Keleş et al., 2018), but others that it has no effect (Kim et al., 2006). It has also been suggested that EMF may exhibit therapeutic properties when used at low doses (Wang et al., 2019; Li et al., 2019).

Some diseases, such as cervical spondylosis and lumbar spinal stenosis, particularly affect the vertebra (Schroeder et al., 2016). Lumbar spinal stenosis is a nebulous entity involving narrowing of the lumbar spinal canal, resulting in compression of the neural elements (Schroeder et al., 2016). Common risk factors are age, lumbar spine surgery, and lumbar spinal canal narrowing (Schroeder et al., 2016). Cervical spondylosis occurs as a result of narrowing of the lumbar spinal canal due to

congenital or facet joint hypertrophy (Wang et al., 2020). This is caused by degenerative changes in the cervical intervertebral disc, posterior joint and bone hyperplasia, and variations of the cervical vertebrae joints (Wang et al., 2020). The possibility of EMF involvement in these pathologies that occur in the vertebra has been raised by some authors (Wang et al., 2020).

The present study particularly focuses on the prenatal period of organ development. The neural tube forms in the embryo in the fourth week after conception and later becomes the baby's spinal cord, spine and brain. The neural tube caudal to the fourth pair of somites develops as the medulla spinalis. The vertebrae also begin to form in the fourth week. Cartilage centers are formed in the vertebrae in the sixth week. In the 12th week of development, the spinal cord begins to change position, while its anatomical structure continues to develop. Bone ossification begins between the sixth and seventh weeks of embryonic development and continues until the age of approximately 25 years (Moore and Persaud, 1993). In rat pups, the neural tube forms in the embryo in the fourth week (gestational days 10.5–11) after conception.

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The spinal cord begins to develop on gestational day 16, and the spine and brain on gestational day 13.5 (Rice and Barone, 2000). This study was planned in the light of these data.

Similarly to other nervous system structures, the development of the vertebrae is influenced by various factors (Au et al., 2010; Wallingford et al., 2013). Since the spinal cord is particularly sensitive in the prenatal period, the normal morphology of the vertebrae may be compromised by a number of internal and external environmental factors (Au et al., 2010; Wallingford et al., 2013). EMF has been proposed as one such factor. Our scan of the literature revealed no previous studies of the effects of EMF on the vertebrae. However, previous studies have shown that nervous system structures are affected by EMF (Baş et al., 2013; Odacı et al., 2008; Odacı et al., 2013; İkinci et al., 2013). This suggests that EMF may exhibit effects on the vertebrae when applied in the prenatal period.

We encountered no previous studies investigating the postnatal effects of EMF applied in the prenatal period. This study was therefore planned around EMF exposure in the embryonic period, and evaluated the vertebrae of the subsequently born rat pups. Since spinal cord development continues after delivery, 32-day-old rat pups were included in the study.

2. Materials and methods

2.1. Experimental procedures

Experimental procedures were approved by the Institutional Ethics Committee of Niğde Ömer Halisdemir University, Turkey (Protocol no. 2019/34 dated 14.11.2019). The research was carried out in accordance with the principles of the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

Rats used in the experiment were obtained from the KTU Surgical Application and Research Center. Sixteen female Sprague Dawley rats weighing 180–250 g exhibiting regular cycles were mated with male rats. The following day, vaginal smears were used to identify the pregnant animals, and that day was regarded as day 0. Six pregnant rats were randomly selected and included in the study. Rats were divided into two groups, a control group ($n = 3$) and an EMF group ($n = 3$). The control

group was not subjected to any EMF application. EMF group rats were exposed to 900 MHz (21.1 T and 2.11×10^{17} G) EMF for 1 h at the same time every day starting from the 13th day of pregnancy. The six pregnant rats gave birth to 23 female offspring (control group 13 and EMF group 10). Twelve pups were used in the study. These were divided into a pup EMF group (PEMF) ($n = 6$) and pup control group (PC) ($n = 6$). No procedure was performed on the pup rats postnatally.

SAR values resulting from cellular phone exposure are based on altering the differential forms of Maxwell's equations with central difference equations and digitizing them in time and location on SEMCAD X simulation software using the Finite Differences in Time method (Finite Difference Time Domain) (Yee, 1996). In studies related to this, its effect on 900 MHz cell phone SAR values has been simulated as 0.97σ (S/m) in adults, 1.1252σ (S/m) in children Tüysüz and G.A. (2011) and 1.1σ (S/m) in animals (Peyman et al., 2001).

2.2. EMF exposure system

Studies investigating the effects of EMF on the vertebrae. For this reason, the lowest level of EMF, 900 MHz, was employed in this study. The exposure time was planned on the basis of the embryonic period. The EMF application system was installed and applied as described elsewhere (Keleş et al., 2018, 2019a; Keleş et al., 2019b). This system has been employed in many previous studies (Odacı et al., 2016; İkinci et al., 2016; Aslan et al., 2017). EMF was measured from nine different previously determined points on the inner floor of the cage (C.A 43 Isotropic Electrical Field Intensity Meter, Chauvin Arnoux Group, Paris, France) (Fig. 1).

2.3. Body weight analysis

Rats in all groups were weighed using a sensitive digital scale (KERN & SOHN GmbH, D-72336 Balingen, Germany) before sacrifice.

2.4. Histological procedures

Rat pups were sacrificed by decapitation under deep

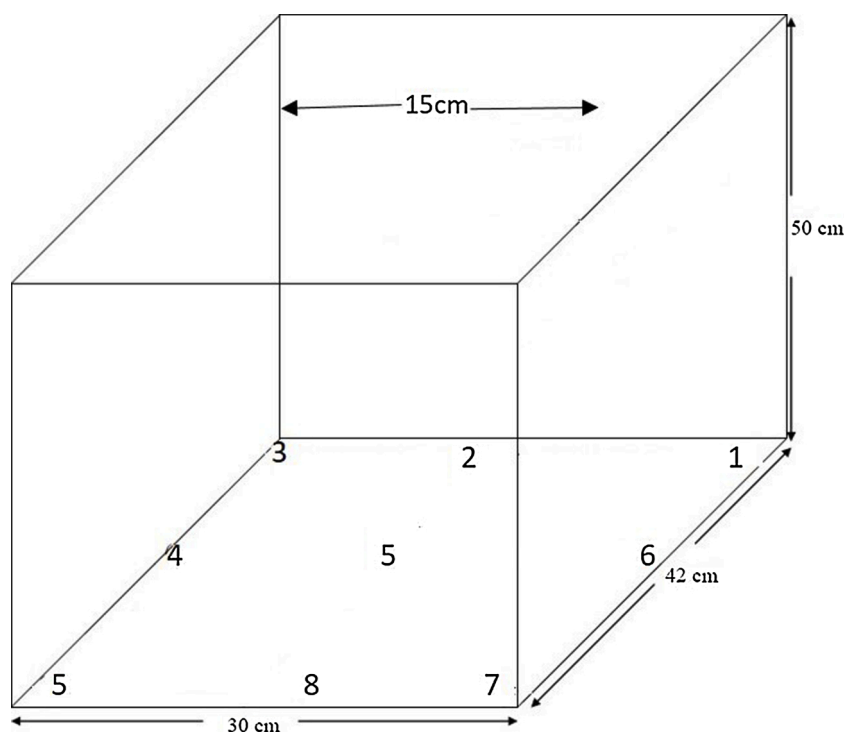


Fig. 1. The position points used to measure EMF intensity on the cage floor (Keleş et al., 2019b).

anesthesia (Ketalar 50 mg/kg) on day 32. The upper thoracic region of the spinal cord and vertebrae were harvested from each animal. The tissues were kept in 10 % formaldehyde overnight and subjected to decalcification in formic acid (5 mL), 10 % formalin (5 mL), and distilled water (90 mL) (Boncroft, 1996). At the end of the decalcification process, the tissues were kept in running water overnight and were then subjected to routine histological procedures. Five-micron-thick transverse sections were taken from the vertebral tissues and stained with hematoxylin Harris (Product Code: HEMH-OT-100, Biognost) and Eosin Y (Product Code: HST-EOQ-0500, Histoplus) for histological evaluation. Six sections were taken from the vertebrae of each rat in all groups (36 sections per group). These stained sections were evaluated under a light microscope (Olympus, BX53, digital camera: DP 80, Olympus and cell-Sens standard software version 1.17, Japan).

2.5. Central canal diameter and area measurements

In order to calculate central diameter and area measurements 10-micron-thick transverse sections were taken from the vertebral tissues and stained with cresyl violet for stereology evaluation. Ten sections (from each of the 10 sections previously selected using systematic random sampling [SSF = 1/10]) were taken from the vertebrae of each rat in all groups (60 sections per group) (Table 1). The areas and diameters of the central canals were measured under $\times 40$ magnification (Fig. 2) (stereological analysis system) in the sections stained with cresyl violet.

The stereological analysis system consisted of a computer and a computer-controlled motorized specimen stage (Prior ProScan, USA), camera (JVC, Japan), microscope (Leica DM4000 B, Germany), a CCD digital camera (JVC, Japan), and an electronic microcator (Heidenhain, Germany). The entire system was designed by Stereoinvestigator software (Version 9, MicroBrightField Inc., USA).

2.6. Statistical analysis

Data distributions were assessed using the Shapiro-Wilk test and a histogram chart. The *t*-test was applied since the groups were normally distributed. Data were expressed as mean $\pm$ standard deviation, and *p* values <0.05 were considered statistically significant. Statistical analysis was performed on SPSS v.22 statistical software. According to the results obtained from the study, power analyzes were made with the Statistical power analysis using G*Power 3.1: Tests for correlation and regression analysis (Faul et al., 2009).

3. Results

3.1. Measurements of EMF findings

Measurements showed that the EMF group pregnant rats were exposed to an average electric field intensity of 13.77 V/m (0.50 W/m²) within the exposure cage. The whole body specific absorption rate (SAR) was calculated as 0.025 W/kg (RadHaz SAR Equivalency Calculator, Version 1.0, ©2000 Richard Tell Associates Inc., WA, USA).

Table 1
Stereological analysis strategy.

Stereological Analysis parameters	Values
Section sampling fraction	1/10
Sampled sections from each spinal cord	10
Paraffin section thickness (μm)	10
Section thickness for safe range of measurements (min–max) (μm)	6–8
Objective lens for thickness calculation (immersion oil was used)	$\times 40$

3.2. Body weight results

Statistical comparisons of the mean body weights of the study groups revealed no significant differences between PC and PEMF (*p* = 0.530). Mean body weight values obtained on the 32nd day were 59.58 ± 2.87 g in the PC group and 60.58 ± 2.44 g in the PEMF group (Table 2) (Fig. 3).

3.3. Histopathological evaluation of vertebrae

Transverse sections from the PC group exhibited normal vertebrae, while the PEMF group exhibited loss of cartilage tissue in the vertebral body and conversion into bone tissue (Fig. 4). A small amount of cartilage tissue was observed in the vertebrae outside the vertebral body in the PEMF group (Fig. 5).

3.4. Central canal measurement results

The *t*-test results indicated statistically significant increases in the central canal area in the PEMF group compared to the PC group (*p* = 0.001) (Fig. 6) (Table 2). Comparison of the central canal diameter between the PEMF group and the PC group also revealed a statistically significant increase in the PEMF group (*p* = 0.000) (Table 2) (Fig. 7).

3.5. Area, diameter and weight power analysis results

Since there is no reference study for the features examined in this study, power analyzes were made according to the results of the study. According to the results obtained from the study, area, diameter and weight power analyzes were determined (power 0.99, 0.99 and 0.15 respectively). The number of animals required for the test to be power 0.90 was checked. It was determined that 122 animals should be used. The use of this number of animals is not considered ethical.

4. Discussion

The results of this study revealed histological changes in the PEMF group vertebrae. Histological examination revealed that PEMF group vertebral body lost cartilage tissue and was converted into osseous tissue. A small amount of cartilage tissue was also observed in the vertebrae outside the vertebral body in the PEMF group. The vertebral body articular surfaces are covered by cartilage (hyaline cartilage) and are connected by the intervertebral disc. The vertebral body articular cartilage is designed for weight-bearing (Colombier and Risbud, 2018). In the present study, this cartilage structure was transformed into bone. We attributed this to EMF exhibiting a stimulating effect on osseous tissue. Some previous studies have reported that EMF increases bone maturation, bone volume, and osseous trabeculation (Tabrah et al., 1998; Nisbet et al., 2016) and stimulates osteoblasts (Wiesmann et al., 2001). However, other previous studies have reported no effect of EMF on bone tissue (Ryang et al., 2013). Other studies have also reported that EMF applied at different intensities reduced bone mineral density (Yildiz et al., 2006) and had a significantly adverse effect on bone fracture healing (Aslan et al., 2013). One study involving prenatal application of EMF observed adverse impacts on bone (femur, tibia, and ulna) tissue development (Erkut et al., 2016). However, the vertebrae have not previously been investigated. Our results suggest that EMF may have a stimulating effect on bone tissue, and that bone tissue may increase as a result of 900 MHz EMF administration in the prenatal period.

Another possibility is that EMF impairs cartilage manufacture in the prenatal period. Bone tissue may have replaced cartilage tissue in the present study. In agreement with our own results, one previous *in vitro* study reported that pulsed EMF therapy reduced expression of genes associated with disc degeneration in human intervertebral disc cells (Miller et al., 2016). However, those authors also reported that the application of pulsed EMFs enhanced the paracrine function of mesenchymal cells in terms of cartilage regeneration (Parate et al., 2020;

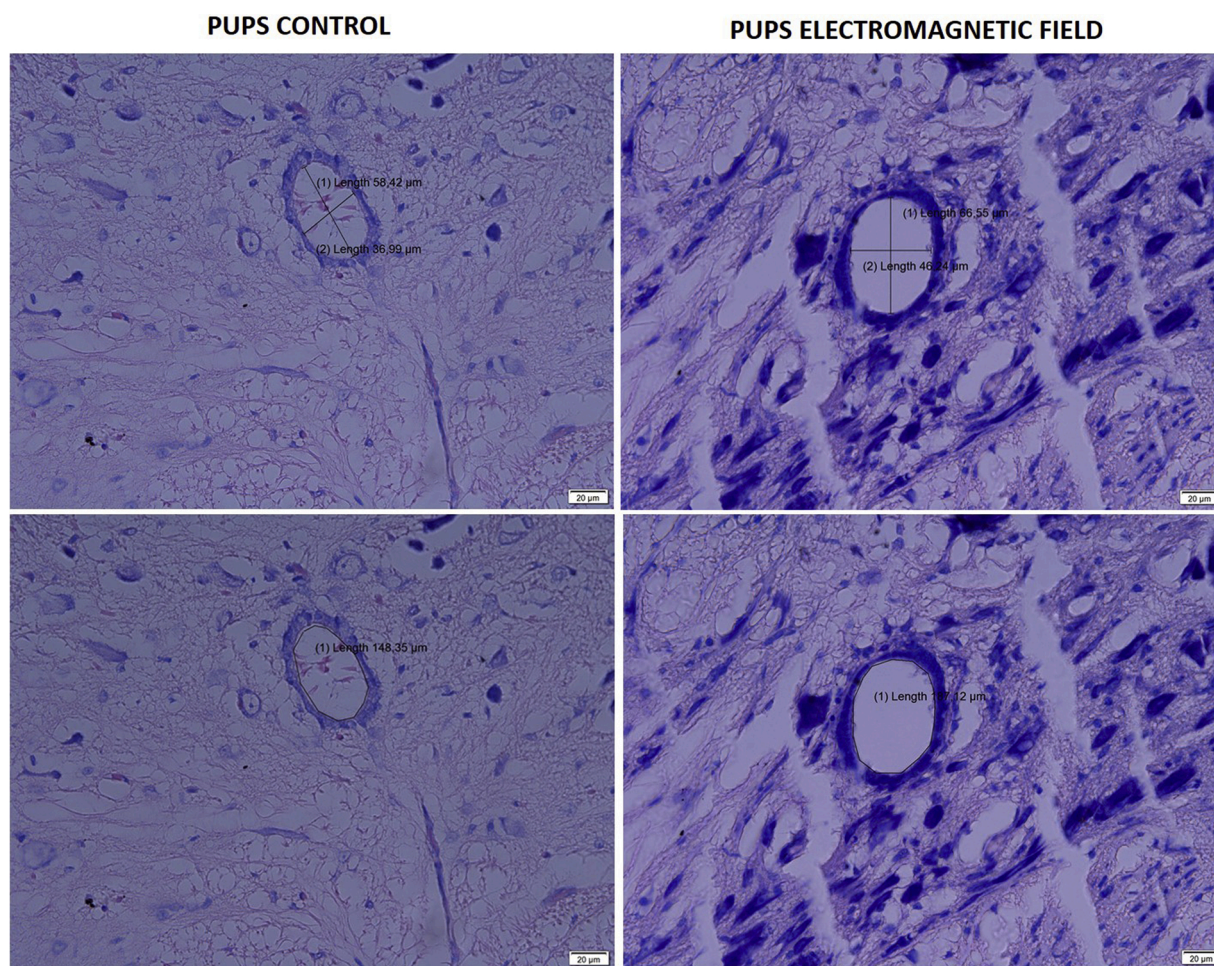


Fig. 2. Representative micrographs of central canal diameter and length measurements from control and electromagnetic field group pups ($\times 40$, cresyl violet). The soft tissue around the central canal is the spinal cord.

Table 2

Analysis of central canal areas and diameters in the control and electromagnetic field group pups.

	Pups Control (n = 6) Mean $\pm$ Standard Deviation	Pups EMF (n = 6) Mean $\pm$ Standard Deviation	P value
Area (m ²)	150.24 $\pm$ 7.64	198.12 $\pm$ 17.86	0.001*
Diameters (μ m)	46.48 $\pm$ 3.20	63.00 $\pm$ 6.65	0.000*
Body weights (g)	59.58 $\pm$ 2.87	60.58 $\pm$ 2.44	0.530

EMF: Electromagnetic field. (*) Indicates a significant difference between groups.

Stefani et al., 2020). The effects on cartilage tissue of pre- and postnatal EMF application are still unclear. Our review of the literature revealed no previous studies investigating the effects on cartilage tissue in rat pups born after prenatal EMF exposure. The decreased cartilage tissue observed in this study suggests that EMF applied in the embryonic period may suppress the development of cartilage cells. Bone tissue may have increased as a result of cessation or regeneration of cartilage tissue.

The structural changes occurring in the vertebrae may also be present in the disc structure. Disc degenerations are progressive changes that affect the spine and that result from various factors including old age, smoking, obesity, and incorrect exercise (Navone et al., 2017; Kadow et al., 2015)). EMF is thought to constitute another of these factors. One study reported that EMF therapy reduced gene expression in disc degeneration (Miller et al., 2016). Another study observed cellular proliferation stimulation in human intervertebral disc cells after EMF

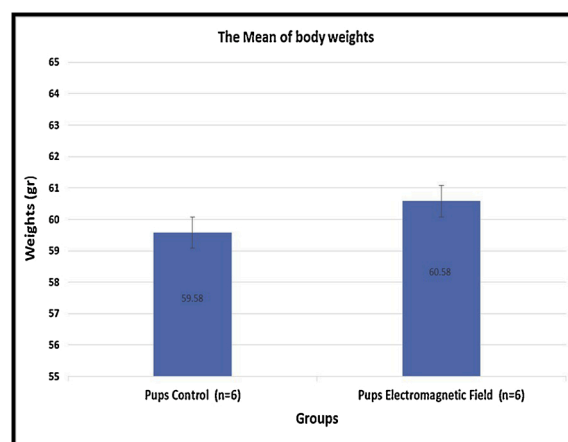


Fig. 3. Representative chart showing the mean of body weights in the pup control (n = 6) and pup electromagnetic field groups (n = 6). There is no statistical difference between the groups.

exposure (Lee et al., 2010). Since the effects of EMF on the disc were not examined in the present study, no definite information was yielded concerning disc degeneration. However, we think that EMF may cause disc degeneration and affect the vertebrae in this location.

Another important finding from the present study is that expansion was observed in the central canal in the PEMF group. Contrary to

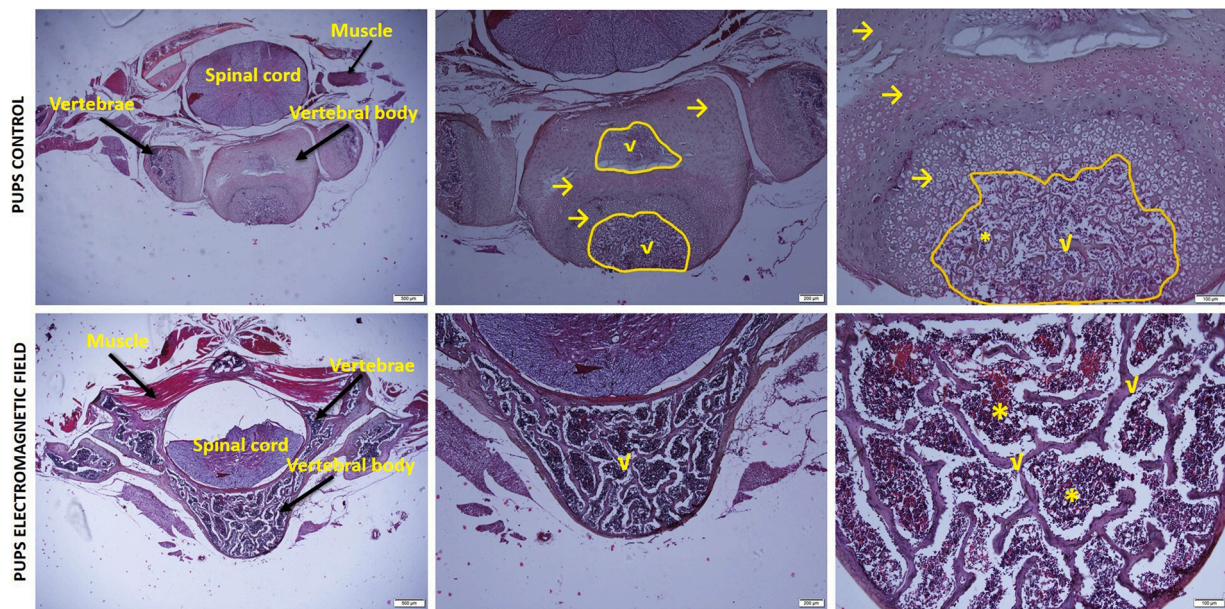


Fig. 4. Representative micrographs of control and electromagnetic field group pup spinal cords ($\times 2$, $\times 4$, $\times 10$, Hematoxylin & Eosin). (→) cartilage tissue, (✓) trabecula and scratched area bone tissue, (*) bone marrow.

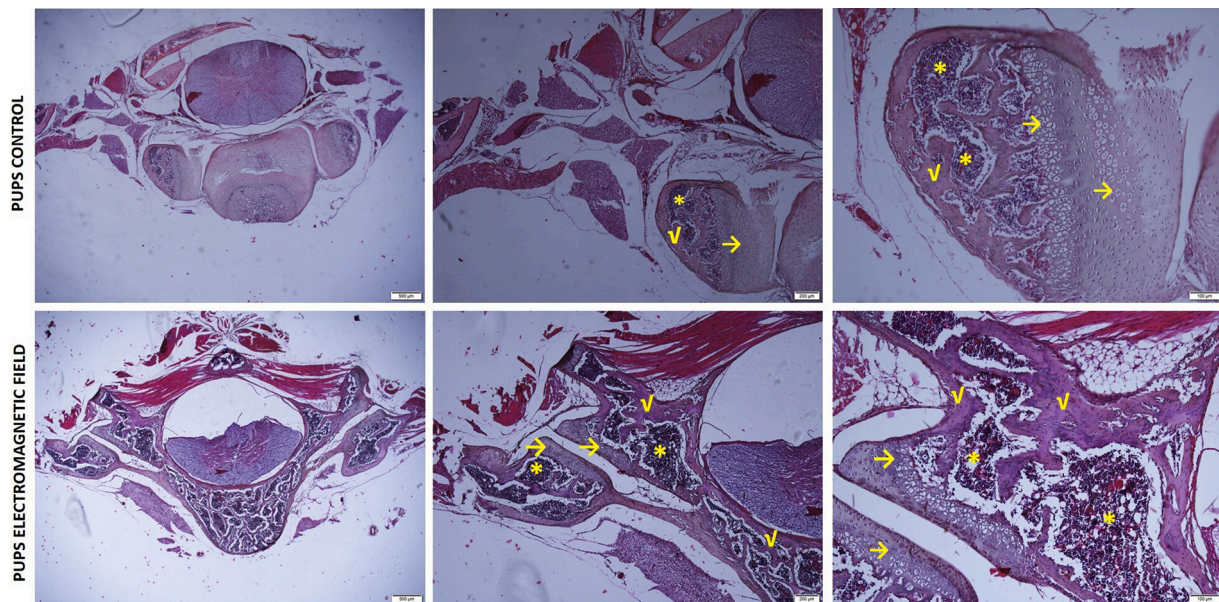


Fig. 5. Representative micrographs of the lateral parts of the spinal cords of pups from the control and electromagnetic field groups ($\times 2$, $\times 4$, $\times 10$, Hematoxylin & Eosin). (→) cartilage tissue, (✓) trabecula, (*) bone marrow.

expectations, expansion occurred in both the central canal diameter and area in the PEMF group. One previous study reported that dilation occurred in the central canal as a result of spinal cord injury (Radojicic et al., 2007). However, there is no information in the literature regarding the effect of EMF on the central canal. However, previous studies have reported deleterious effects of EMF on central nervous system soft tissues (Odaci et al., 2013, 2016; İkinici et al., 2016). We think that the same effect may be involved in the central canal.

Body weight is an important indicator of the health status of the organism. The spinal cord, which keeps the body in a state of balance, is particularly affected by weight changes. Based on our data, exposure to 900 MHz EMF during the prenatal period had no effect on the weight of the female pup rats. At the same time, these results showed that weight had no effect on central canal expansion and vertebrae structural

changes in the spine of the female pup rats.

The findings of the present study indicate that exposure to a 900-MHz EMF in the prenatal period adversely affected the female pup rat vertebrae. Cartilage tissue in vertebrae decreased in the PEMF group and was converted into bone tissue, also leading to enlargements in the central canal. These findings now raise the question of whether EMF impacts on the development of neurological and neurosurgical diseases. We encountered no other studies on this subject. Further studies involving areas such as immunohistochemistry, immunofluorescence, biochemistry, and stereology are now needed to investigate this emerging picture.

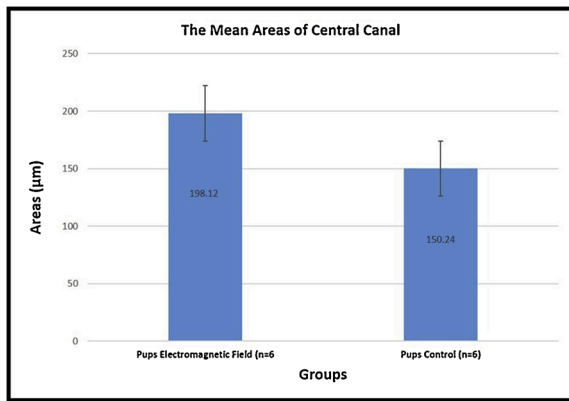


Fig. 6. Representative chart showing the central canal areas in the pup control (n = 6) and pup electromagnetic field groups (n = 6). Different letters (a, b) denote significant differences between the groups.

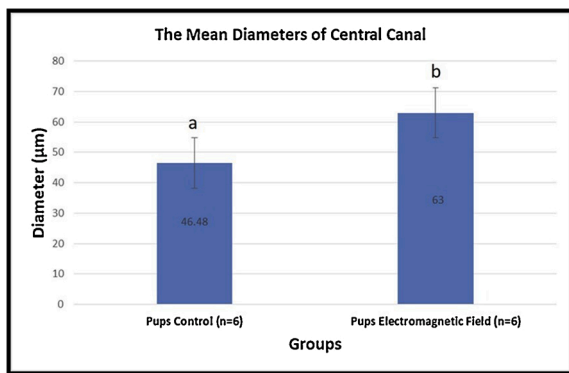


Fig. 7. Representative chart showing the central canal lengths in the pup control (n = 6) and pup electromagnetic field groups (n = 6). Different letters (a, b) denote significant differences between the groups.

Declaration of Competing Interest

The authors report no declarations of interest.

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