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Effect of 6 GHz radiofrequency electromagnetic field on the development of fetal bones

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ABSTRACT

This study examined the impact of 6 GHz (0.054 W/kg SAR) Radiofrequency-Electromagnetic Field (RF-EMF) on prenatal bone development. In this study, 20 female and 20 male Wistar Albino rats divided into four groups. The Control group received no treatment, while in Group-I, only male rats were exposed to RF-EMF, female rats had no exposure. Group-II, both male and female rats received RF-EMF treatment. While in Group-III, only female rats were exposed to RF-EMF, male rats had no exposure. The exposure lasted 4 hours per day for 6 weeks. The rats were then allowed to mate within the group. After pregnancy, pregnant rats (Group-II and III) were exposed 4 hours per day for 18 days. On the 18th day of gestation, fetuses were removed and their weight and various lengths were measured. The skeletal system development of fetuses was examined with double skeletal staining method and assessed ossification in the extremities. In the study, fetal weights, head-tail length, occipital-frontal and parietal-parietal lengths significantly increased in all exposure groups when compared to the control group ($p < 0.001$). Although occipital-frontal length was smallest in Group-I, Group-II and Group-III were more higher than the control group ($p < 0.001$). The bones of the anterior and posterior extremities showed significant increases in length, ossification zone length, and ossification percentage in all experimental groups compared to the control group ($p < 0.001$). Our study showed that rats exposed to 6 GHz (0.054 W/kg) RF-EMF during the prenatal period had significant increases in bone development.

PLAIN-LANGUAGE SUMMARY

Radiofrequency Electromagnetic Field (RF-EMF) sources are one of the most widely used technology systems in daily life. This study examined the impact of 6 GHz RF-EMF on prenatal bone development.

In this study, 20 female and 20 male Wistar Albino rats divided into four groups. The control group rats received no treatment, while in Group-I, only male rats, Group-II, both male and female rats, while in Group-III, only female rats were exposed to RF-EMF. The exposure lasted 4 hours/day for 6 weeks. The rats were then allowed to mate within the group. After pregnancy, pregnant rats (Group-II and III) were exposed 4 hours/day for 18 days. On the 18th day of gestation, fetuses were removed 10 fetuses each group were randomly selected in each group and their weight and various lengths were measured. The skeletal system development of fetuses was examined with double skeletal staining method. The ImageJ program was used assess ossification in the extremities.

In the study, fetal weights, head-tail, occipital-frontal and parietal-parietal lengths significantly increased in all exposure groups when compared to the control group ($p < 0.001$). Although occipital-frontal length was smallest in Group-I, Group-II and Group-III were more higher than the control group ($p < 0.001$). The bones of the anterior and posterior extremities showed significant increases in length, ossification zone length, and ossification percentage in all experimental groups compared to the control group ($p < 0.001$). Our study showed that rats exposed to 6 GHz (0.054 W/kg) RF-EMF during the prenatal period had significant increases in bone development.

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6 GHz RF-EMF; prenatal; ossification; extremity

Introduction

The rapid advancement of telecommunication technologies has addressed user needs more quickly and effectively and therefore interest in these systems has increased. However, the widespread utilization of these products also brings about inevitable exposure to radiation. The lack of agreement on how to evaluate the health and safety risks associated with exposure to RF-

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EMF sources in varying frequencies has led to increased public concern (Karipidis et al. 2021; Velghe et al. 2021).

The effects of RF-EMF on living organisms are determined by the intensity, duration of exposure and frequency of the electromagnetic waves. Exposure to multiple RF-EMFs simultaneously can cause heat increase in tissues (Majchrzak et al. 2008). The Specific Absorption Rate (SAR; W/kg) is used to measure the energy absorbed from RF-EMFs by the body. Increased RF-EMF exposure from new technologies may impair normal cell differentiation and proliferation rates at the macromolecular level (Christopher et al. 2021). It is important to establish safe limits for EMF exposure in terms of frequency and duration for humans (ICNIRP 2020). In May 2011 the International Agency for Research on Cancer (IARC) classified Radiofrequency Radiation (RFR) as a potential "Group 2B carcinogen" for humans due to reports on the potential carcinogenic effects of RF-EMF exposure (Hardell 2017).

Several studies have shown that RF-EMFs affect bone formation and structure in different ways. These are physical-physiological and cellular-molecular processes as well as their effects on bone metabolism and stimulation of osteogenesis (Chang and Chang 2003; Ciombor et al. 2002; Emre et al. 2021). In bone development before adulthood, construction events have predominance over destruction, and thus it is established a balance that continues into adulthood and throughout life for maintaining a normal bone structure. This balance is achieved through the coordination of osteoblasts and osteoclasts in bone activities (Kong et al. 2000).

Exposure of pregnant women to EMFs may reduce amniotic fluid and increase the risk of spontaneous abortion and death. Exposure to RF-EMF during pregnancy can also affect growth and development of the fetus (Cordelli et al. 2023). Exposure to EMFs increased the risk of abortion in pregnant rats and reduced the weight and body length of the fetus (Irani et al. 2023). Exposure to EMFs during the prenatal period can be associated with congenital anomalies and fetal developmental/neurodevelopmental disorders (Kashani et al. 2023).

In the literature, RF-EMF applications on biological tissues have generally been studied in the sub-6 GHz range such as 2.45 GHz and 1800 MHz and the sub-6 GHz frequency band is the most widely utilized in the 5th generation mobile network technologies (Lin et al. 2024). In our study, we used 6 GHz RF-EMF because it is a first in the literature and it is the boundary value between sub-6 GHz and mmWages category. Moreover, we focused on the effects of high-frequency RF-EMFs because of the growing use of new technological products. In our study, we examined the effects of 6 GHz RF-EMF exposure on the fetal development and ossification of prenatal fetuses.

Materials and methods

Animals

This study was conducted in accordance with Decision No: 4/11 taken by the Local Ethics Committee of Experimental Animals of Cukurova University. 20 adult female Wistar Albino rats weighing between 200–250 g and 20 adult male Wistar Albino rats weighing between 250–300 g were used. These rats were bred at the Health Sciences Experimental Application and Research Center. Throughout the experiment, the rats were housed in a laboratory with a temperature of 24 ± 2°C and a humidity of 40–60%. They were kept in line with the circadian rhythm (12 hours light/dark) and provided with unlimited pellet feed and tap water.

Application setup

In our study, both the control and experimental groups were placed in different two Faraday cages (in size: 100 × 100x90cm) (Figure 1a, b). The Control (Sham) group, Group-I female and Group-III male rats were placed in one of the Faraday cages together with the standard cages in which they lived. Rats in this group were protected from possible electromagnetic radiation in the external environment and were not exposed to any treatment (Figure 1b). The experiment cage had four compartments of equal size. The Group-I male, Group-II male and female and Group-III female rats were exposed to RF-EMF and had the option to seek shelter in the cage (Figure 1A). Before RF-EMF application to rats, the monopole antenna coming out of the RF generator placed at the upper midpoint of the Faraday cage was fixed in the middle of the special application cage (ground height: 35 cm) (Figure 1a, c).

Exposure system for RF-EMF

The RF generator (FR6GX-2W, Turkey) used in the application emits a sinusoidal wave with a fixed frequency modulation (5.8–6 GHz) in Continuous Wave (CW) format, with an adjustable power range of 0–2 Watts. The generator requires a power supply of 220 ~ 230 V and operates within a temperature range of −40°C to + 60°C, with a 50 Ω output. CW signal was used in this study. A monopole antenna which consists of a single conductor plate that evenly distributes the RF signal from the generator in all directions was used.

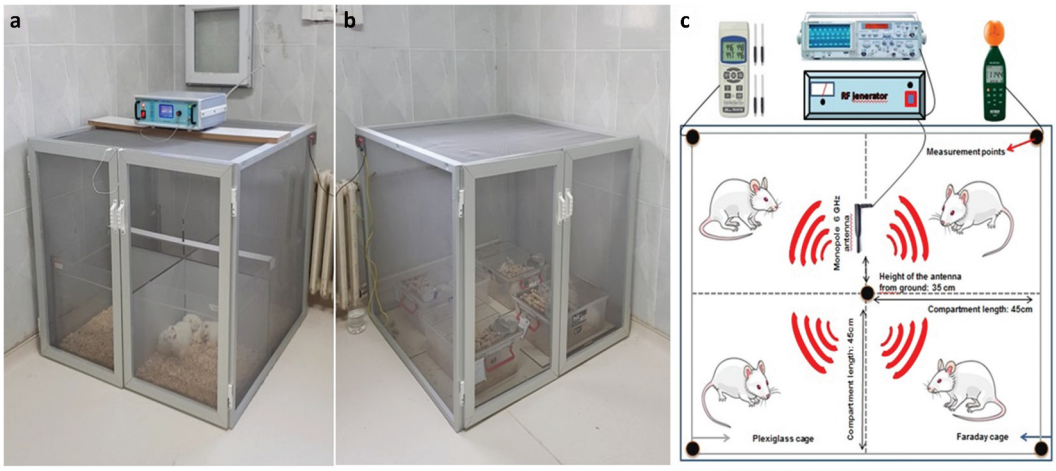


Figure 1. RF-EMF exposure setup. a) experimental setup and RF generator with 6 GHz RF-EMF applied, b) control groups of rats. c) simulation of the top view of the application cage.

Formation of experimental groups

The impact of 6 GHz RF-EMF on prenatal bone development was studied using four groups: Control, Group-I, Group-II, and Group-III. Each group consisted of 10 rats (5 males, 5 females) that were either exposed to RFR (+) or not exposed to RFR (-). Control (males RFR(-), females RFR(-)), Group-I (males RFR(+), females RFR(-)), Group-II (males RFR(+), females RFR(+)) and Group-III (males RFR (-), females RFR(+)).

Specific absorption rate (SAR)

When the RF generator power was operating at 1 Watt, we utilized the ElectroSmog meter (TES-593, Taiwan) which is a portable device to measure the electric field (EF), magnetic field (MF), and power density (PD). We recorded the RF-EMF parameters as follows: EF = 6.29 ± 0.04 V/m, MF = 16.31 ± 0.09 mA/m, and PD = 11.80 ± 0.12 W/cm^2.

In our study, we used the Sim4Life 7.0.2 software, developed by Zurich MedTech AG in Zurich, Switzerland, which is based on the Finite-Difference Time Domain (FDTD) method. To calculate SAR values, we used three-dimensional model simulations of a rat represented as a volumetric pixel (voxel), generated using ViZoo 1.0 (Virtual Zoo) software from Zurich MedTech AG. The simulation was done using the IEE/IEC62704–1 averaging method.

1 g averaged SAR distribution was evaluated. The whole body averaged SAR value for adult rats was 0.083 W/kg (Figure 2a, b), while for prenatal fetuses of pregnant rats, it was 0.054 W/kg (Figure 2c). These SAR values were confirmed by comparing values calculated in existing literature for the same conditions, orientation, and antenna power (Emre et al. 2021; Persia et al. 2018).

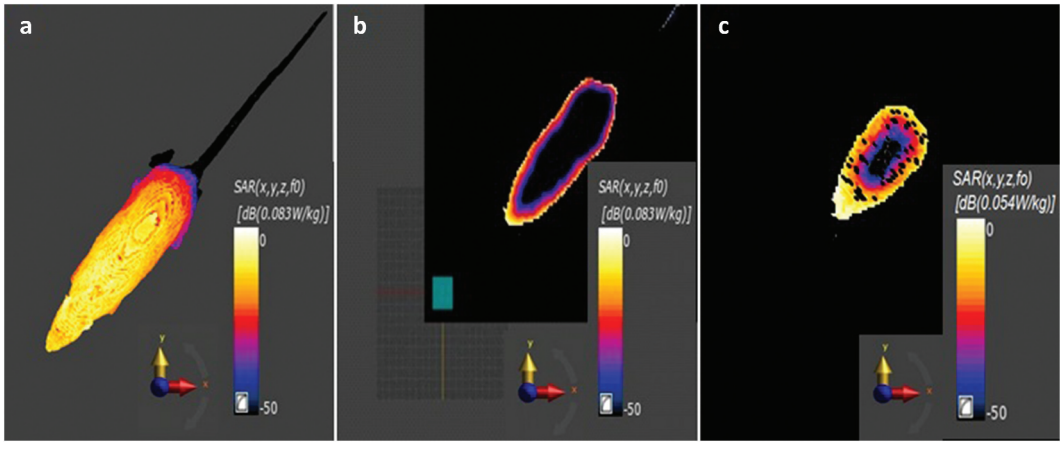


Figure 2. SAR simulations. a) adult rat voxel model. b) SAR distribution in adult rat. c) SAR distribution in pregnant rat with fetus.

Pregnancy case

Following exposure to 6 GHz RF-EMF for 4 hours every day for 6 weeks, the animals in each group were allowed to mate overnight within the group. Vaginal smear examination was performed the next morning and rats with spermatozoa/spermatosomes in their vagina using a flat-type industrial microscope (Nikon Eclipse E200, Japan) were identified and categorized as being 0.5 days pregnant. After pregnancy were identified, pregnant rats (Group II and Group III) that were exposed to RF-EMF were exposed to RF-EMF for 4 hours every day for an additional 18 days. The rats that did not receive any exposure to RF-EMF (Control and Group I) still did not receive any radiation for the next 18 days. All pregnant rats in both control and exposure groups appeared physically healthy.

Prenatal bone studies

Following the completion of gestation procedures (18th day) in female rats, pregnant rats from each group were intraperitoneally anesthetized with xylazine (0.05 mg/kg) ve ketamine (0.1 mg/kg) on the 18th day of gestation and incisions were made in their abdominal region. All fetuses were then extracted from the uterus via cesarean section without causing harm, and the placentas were separated and sacrificed along with the mother rat. After fetuses were immediately removed, they were standardized in their intrauterine positions. 10 fetuses in each group were included in the study and the fetuses of each group were randomly selected from the fetuses of adult pregnant rats in each group. The gender of the fetuses was not evaluated. Fetuses that were damaged during all these procedures or had malformations were not included in the study. The head-tail, occipital-frontal length, and parietal-parietal lengths of each fetus were measured using digital calipers. The weights of all fetuses were then measured using a precision balance.

Double skeletal staining method

Double Skeletal Staining Method is a technique generally used to examine fetal bones in pregnant rats. In this method, the bones and cartilages which form the skeleton stains in different colors or different shades of the same color (Yilmaz 2019). Before procedure with the double skeletal staining protocol, all fetuses were placed in 70% ethyl alcohol for 4–7 days to completely remove the fluid from the fetal tissues. Then, the fetuses were removed from 70% ethyl alcohol and kept in pure acetone for 1–3 days to remove the oil. Following this procedures, their skins were peeled and their eyes and internal organs were removed. The fetuses were removed from acetone and were placed in a double skeletal staining solution consisting of 1 mg/mL Alizarin Red-S (indicates the ossification area) and 3 mg/mL Alcian Blue (indicates the cartilage area) in glass containers. The glass containers containing the fetuses and the solution were covered with cling film to prevent contact with air. They were incubated for 7 days in an oven the temperature of which was set at 38–40°C, and thus the tissues bone were stained. At the end of the 7th day, all fetuses were washed under running tap water for 2 hours to allow them to react with water. After these processes were completed, they were passed to the transparency stages with 1% potassium hydroxide. At this stage, the stained all fetuses were placed in 20%, 50%, and 80% glycerin and finally 100% pure glycerin. Following the transparency stages, the skeletal system of the fetuses was revealed (Figure 3).

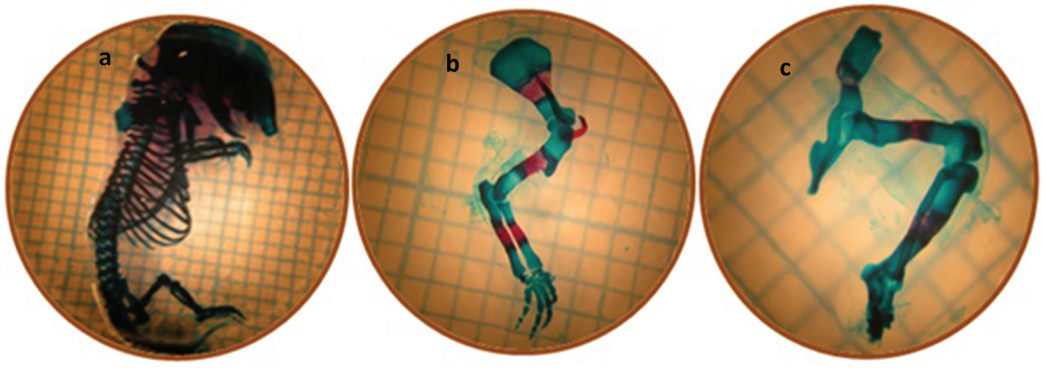


Figure 3. The skeletal system of the fetuses (the areas stained with blue indicate cartilage and red areas show ossification). a) fetal skeletal system, b) anterior extremity bones, c) posterior extremity bones.

Development of extremity bones

The joints of the extremity bones in all groups were separated using a microscope (Zeiss, Stemi 2000CS, Germany). Nikon E5700 camera was mounted to the microscope to photograph the bones placed on millimeter paper. The measurements of the bone and cartilage areas were taken using the ImageJ software program. Following calibration, bone measurements were manually determined using the Wand Tool feature in ImageJ. The results were recorded in millimeters. The measurements were carried out on the scapula, humerus, radius, and ulna in the anterior extremity and on the femur, tibia, and fibula in the posterior extremity. A total of 20 extremity bones from 10 fetus each of group, both right and left sided, were examined. It was observed that ossification had began in all extremity bones (Figure 4).

Statistical analysis

In the study, normality of continuous variables was evaluated using the Shapiro-Wilk test. Numerical measurements were summarized as mean and standard deviation (median and minimum-maximum where necessary). The One-Way ANOVA test was conducted to compare more than two normally distributed groups, followed by the Tukey test for multiple comparisons. The data was analyzed using IBM SPSS Statistics Version 20.0 package program (SPSS reference: IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0 Armonk, NY: IBM Corp.). A statistical significance level of 0.05 was used for all tests.

RESULTS

Growth parameters

After removing all fetuses from pregnant rats in each group, the total head-tail, occipital-frontal, parietal-parietal lengths, and weight of each fetus were measured (Table 1). The mean total head-tail, occipital-frontal, parietal-parietal lengths were the highest in Group-3. The mean fetal weight was the highest in Group-I. The mean total head-tail, parietal-parietal lengths of fetuses were the lowest in Control Group. The mean occipital-frontal length of fetuses was the lowest in Group-I. Statistically significant differences were found in all measured parameters between the control group and the exposure groups ($p < 0.05$).

Morphological measurements of extremity bones development

The total bone length, the length of ossification areas and the percentage of ossification were calculated for all extremity bones. The ossification percentage was calculated as following formula: (Ossification Surface Area/ Total Bone Surface Area) x100. Data obtained from

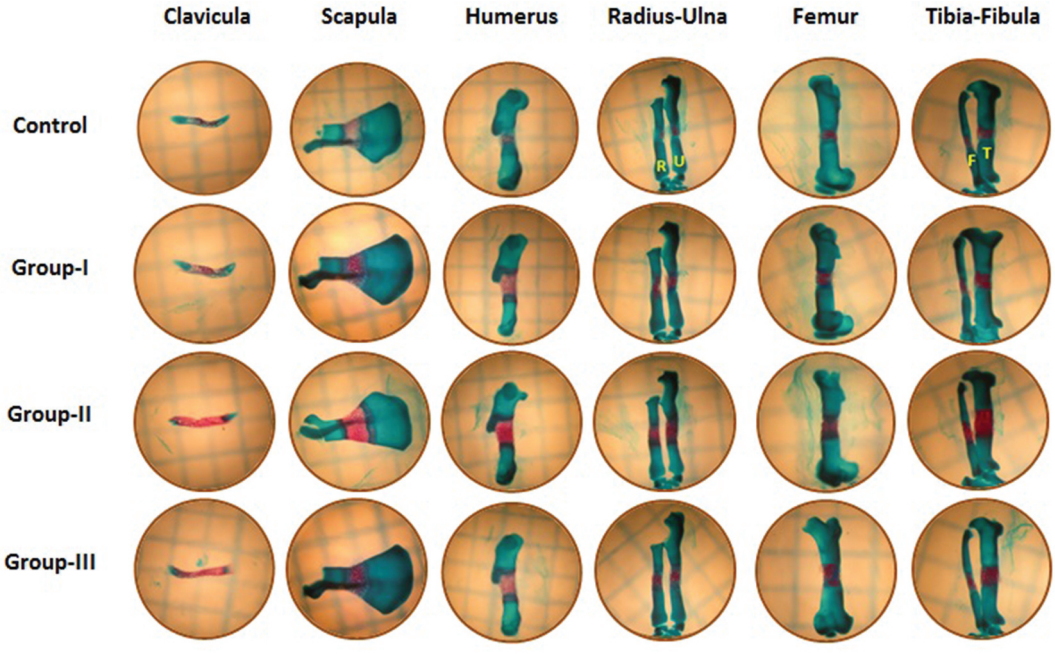


Figure 4. Images of the anterior and posterior extremity bones (the areas stained with blue indicate cartilage and red areas show ossification).

Table 1. Distribution of mean growth parameters of fetuses in the prenatal period.

Measurements	N	Groups: Control Mean±SD (Min-Max)	Group-I Mean±SD (Min-Max)	Group-II Mean±SD (Min-Max)	Group-III Mean±SD (Min-Max)	P*
Head-Tail length (mm)	10	$23.91 \pm 0.72^{b,c,d}$ (23.1–25.5)	25.28 ± 0.93^{a} (23.2–26.3)	25.08 ± 0.90^{a} (24.0–26.7)	25.62 ± 0.48^{a} (24.9–26.6)	**0.000**
Occipital-Frontal length (mm)	10	$7.80 \pm 0.18^{c,d}$ (7.56–8.14)	$7.73 \pm 0.17^{c,d}$ (7.45–7.96)	$8.16 \pm 0.25^{a,b}$ (7.75–8.42)	$8.28 \pm 0.36^{a,b}$ (7.43–8.72)	**0.000**
Parietal-Parietal length (mm)	10	6.23 ± 0.18^{d} (6.03–6.61)	6.53 ± 0.24 (6.05–6.81)	6.56 ± 0.33 (6.17–7.05)	6.78 ± 0.33^{a} (6.25–7.13)	**0.001**
Fetus weights (g)	10	$1.30 \pm 0.08^{\dagger b,c,d}$ (1.18–1.42)	1.50 ± 0.09^{a} (1.32–1.63)	1.43 ± 0.07^{a} (1.30–1.54)	1.49 ± 0.08^{a} (1.33–1.57)	**0.000**

*.One-Way ANOVA; $p < 0.05$ was considered statistically significant.
†.a:Significant when compared with the control group.b:Significant when compared with group-I.c:Significant when compared with group-II.d:Significant when compared with group-III.

morphometric measurements were then compared between all groups (Table 2). The mean total bone length, length of ossification areas and ossification percentage were highest in Group-II. These parameters in Group-I and Group-III differed according to the extremities. The mean total bone length, length of ossification areas and ossification percentage were lowest in Control Group. When the anterior and posterior extremity bones of the control group were compared with the other exposure groups, it was determined that the total bone length was shorter, the ossification area was narrower and the ossification percentage was smaller. According to our data, it can be concluded that prenatal exposure to 6 GHz RF-EMF before pregnancy and during pregnancy has a significant impact on the development of the fetal bones.

Discussion

With technological products being used globally, it is nearly impossible to find individuals who have not been exposed to radiation emitted from these systems. In our research, we investigated the effects of 6 GHz (0.054 W/kg SAR) RF-EMF on fetal bone development. When we compared total weight, head-tail, occipital-frontal and parietal-parietal lengths of fetuses in all exposure groups with the control group data, we found a statistically significant increase in the relevant characteristics in the exposure

Table 2. Distribution of anterior and posterior extremity bone data between groups.

Measurements	Bones	Groups: Control ($n = 20$) Mean±SD	Group-I ($n = 20$) Mean±SD	Group-II ($n = 20$) Mean±SD	Group-III ($n = 20$) Mean±SD	P*
Total Bone Length (mm)	**Clavicula**	$2.28 \pm 0.14^{c,d}$	$2.32 \pm 0.17^{c,d}$	$2.67 \pm 0.20^{a,b}$	$2.63 \pm 0.16^{a,b}$	**0.000**
	Scapula	$3.79 \pm 0.18^{b,c}$	$4.04 \pm 0.17^{a,d}$	$4.15 \pm 0.21^{a,d}$	$3.88 \pm 0.19^{b,c}$	**0.000**
	Humerus	$4.05 \pm 0.25^{b,c}$	4.21 ± 0.18^{a}	$4.35 \pm 0.15^{a,d}$	4.08 ± 0.16^{c}	**0.000**
	Radius	$2.82 \pm 0.20^{b,c,d}$	$3.04 \pm 0.14^{a,c}$	$3.20 \pm 0.18^{a,b,d}$	$3.05 \pm 0.16^{a,c}$	**0.000**
	Ulna	3.83 ± 0.23^{c}	3.98 ± 0.20^{c}	$4.27 \pm 0.20^{a,b,d}$	3.99 ± 0.22^{c}	**0.000**
	Femur	$3.26 \pm 0.17^{b,c,d}$	3.67 ± 0.19^{a}	$3.80 \pm 0.22^{a,d}$	$3.53 \pm 0.15^{a,c}$	**0.000**
	Tibia	$2.98 \pm 0.16^{b,c,d}$	$3.26 \pm 0.29^{a,c}$	$3.45 \pm 0.17^{a,b,d}$	$3.27 \pm 0.23^{a,c}$	**0.000**
	Fibula	$2.74 \pm 0.18^{b,c,d}$	$3.02 \pm 0.21^{a,c}$	$3.27 \pm 0.17^{a,b,d}$	$3.01 \pm 0.23^{a,c}$	**0.000**
Length of Region Exhibiting Ossification (mm)	**Clavicula**	$1.34 \pm 0.13^{b,c,d}$	$1.54 \pm 0.12^{a,c,d}$	$2.34 \pm 0.12^{a,b,d}$	$1.85 \pm 0.16^{a,b,c}$	**0.000**
	Scapula	$1.14 \pm 0.09^{b,c,d}$	$1.34 \pm 0.10^{a,c,d}$	$1.48 \pm 0.10^{a,b,d}$	$1.27 \pm 0.10^{a,b,c}$	**0.000**
	Humerus	$1.16 \pm 0.08^{b,c,d}$	$1.41 \pm 0.14^{a,c}$	$1.64 \pm 0.10^{a,b,d}$	$1.32 \pm 0.12^{a,c}$	**0.000**
	Radius	$0.86 \pm 0.08^{b,c,d}$	$1.05 \pm 0.09^{a,c}$	$1.25 \pm 0.09^{a,b,d}$	$1.11 \pm 0.11^{a,c}$	**0.000**
	Ulna	$1.07 \pm 0.11^{b,c,d}$	$1.27 \pm 0.11^{a,c}$	$1.54 \pm 0.11^{a,b,d}$	$1.27 \pm 0.14^{a,c}$	**0.000**
	Femur	$0.75 \pm 0.06^{b,c,d}$	$0.96 \pm 0.09^{a,c}$	$1.15 \pm 0.10^{a,b,d}$	$0.91 \pm 0.06^{a,c}$	**0.000**
	Tibia	$0.73 \pm 0.07^{b,c,d}$	$1.01 \pm 0.10^{a,c,d}$	$1.15 \pm 0.08^{a,b,d}$	$0.93 \pm 0.09^{a,b,c}$	**0.000**
	Fibula	$0.76 \pm 0.11^{b,c,d}$	$1.11 \pm 0.09^{a,d}$	$1.11 \pm 0.10^{a,b,d}$	$0.98 \pm 0.10^{a,c}$	**0.000**
Percentage Ossification Rate (%)	**Clavicula**	$57.17 \pm 4.27^{b,c,d}$	$64.94 \pm 10.73^{a,c}$	$87.31 \pm 6.45^{a,b,d}$	$69.30 \pm 6.65^{a,c}$	**0.000**
	Scapula	$23.02 \pm 2.92^{b,c,d}$	$26.48 \pm 1.82^{a,c,d}$	$32.70 \pm 1.92^{a,b,d}$	$28.87 \pm 2.61^{a,b,c}$	**0.000**
	Humerus	$20.54 \pm 2.64^{b,c,d}$	$29.60 \pm 2.61^{a,c,d}$	$37.12 \pm 1.88^{a,b,d}$	$32.69 \pm 3.50^{a,b,c}$	**0.000**
	Radius	$29.37 \pm 2.88^{b,c,d}$	$32.57 \pm 3.54^{a,c,d}$	$37.38 \pm 2.84^{a,b}$	$36.59 \pm 4.84^{a,b}$	**0.000**
	Ulna	$22.90 \pm 3.59^{b,c,d}$	$30.45 \pm 4.58^{\dagger a}$	31.54 ± 3.86^{a}	30.10 ± 4.65^{a}	**0.000**
	Femur	$16.97 \pm 1.98^{b,c,d}$	22.93 ± 2.49^{a}	$24.55 \pm 1.56^{a,d}$	$21.83 \pm 2.39^{a,c}$	**0.000**
	Tibia	$20.97 \pm 2.55^{b,c,d}$	$28.62 \pm 4.31^{a,c}$	$33.38 \pm 3.19^{a,b,d}$	$28.34 \pm 2.89^{a,c}$	**0.000**
	Fibula	$24.29 \pm 3.97^{b,c,d}$	$34.51 \pm 6.44^{a,d}$	$34.81 \pm 3.54^{a,d}$	$29.73 \pm 1.93^{a,b,c}$	**0.000**

*.One-Way ANOVA; $p < 0.05$ was considered statistically significant.
†.a:Significant when compared with the control group.b:Significant when compared with group-I.c:Significant when compared with group-II.d:Significant when compared with group-III.

groups ($p < 0.05$). Previous research on the effects of RF-EMF on the prenatal period has also reported significant support for fetal bone development, consistent with our study (Ogawa et al. 2009; Tsarna et al. 2019). Berman et al. exposed pregnant mice to 2450 MHz (CW, 28 mW/cm^2) EMF for 100 minutes per day from day 6 to day 17 of their pregnancy and they found a decrease in the weight of the fetuses on the 18th day. Additionally, they observed that exposure to EMF resulted in only minor skeletal changes (Berman et al. 1982). Ogawa et al. found no side effect of 1.95 GHz RF-EMF exposure for 90 min/day on the bone in the offspring of pregnant rats (Ogawa et al. 2009).

Although most studies have found no adverse effects on fetal development from varying levels of exposure to RF-EMFs, there is still a lack of convincing evidence. In our study, we observed a significant increase in total body weight and bone length of fetuses in the exposure groups compared to the control group (Table 1). These findings also have supported our previous study on the effects of 2.45 GHz RF-EMF exposure on rats before pregnancy (Cantürk-Tan et al. 2018). Morever, several studies claimed that EMF may a positive effect on bone development (Berman et al. 1992; Chang and Chang 2003; Yildiz et al. 2006). But, the study conducted by Sangun et al., they showed that 2.45 GHz EMF resulted in growth restriction and delayed puberty in female rats (Sangun et al. 2015). The differences between these studies may be attributed to factors such as the frequency and SAR value of the RF-EMFs, duration of exposure and the influence of environmental stressors. While some studies have suggested a potential health risk associated with RF-EMF exposure, other epidemiologic studies have not found a direct link to pregnancy (Jensh et al. 1982; Shirai et al. 2017). Overall, RF-EMFs generally do not cause significant harm to the development of fetuses during pregnancy.

In this study, we investigated the effects of 6 GHz (0.054 W/kg SAR) RF-EMF on bone development using the double skeletal staining method. This method allows us to visualize the skeletal system and evaluate the level of development in fetuses (Menegola et al. 2001). We found that parental exposure to 6 GHz RF-EMF before pregnancy and during pregnancy led to a statistically significant increase in the total bone length, the length of ossification areas and the percentage of ossification in the all extremity bones of offspring ($p < 0.05$ (Table 2). Our findings are consistent with a prior study that examined the effects of RFR on bone growth using the identical double skeletal staining method (Cantürk-Tan et al. 2018).

We found a limited number of studies that examined the potential impact of RF-EMFs on the skeletal system in literature. When Lee et al. exposed female mice to RF signals (2.0 W/kg SAR) for 45 minutes at 15-minute intervals daily throughout their pregnancies, they did not determined any negative effects on skeletal defects, growth retardation and any changes in head size in 18-day-old prenatal fetuses in the exposure groups (Lee et al. 2009). In another study, pregnant mice were exposed to 900 MHz (0.6–0.94 W/kg SAR) RFR for 6 minutes per day for 5 days during the prenatal stage and differences in fetal development and bone formation were observed in the fetuses of pregnant mice in the exposed groups compared to the control groups (Fragopoulou et al. 2010). Additionally, in several studies which have investigated the effects of prenatal exposure to RF-EMFs of different frequencies on bone development, it has been noted that bone ossification is delayed, there are minimal changes in bone mineral tissue, skeletal abnormalities, and increase in femur length (Akdag et al. 2010; El-Sayed 2011).

Conclusion

Our study showed that rats exposed to 6 GHz (0.054 W/kg) RF-EMF during the prenatal period had significant increases in bone development. It is premature to conclude that only 6 GHz RF-EMF is solely responsible for these increases. Most studies examining the effects of RF-EMF exposure on bone tissue suggest that it does not cause any harmful effects. Further research is needed to understand the mechanisms by which RF-EMF sources affect bone tissue, including biophysical, biomechanical, and histopathological studies. We believe that our study can contribute to future research on the impact of RF-EMF exposure on bone development and potentially lead to the discovery of alternative treatment methods in the field of biomedicine.

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The authors declare that they have no conflict of interest.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Ethical approval

The study followed the guidelines of Decision No: 4/11, issued by the Çukurova University Animal Experiment Local Ethics Committee.

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