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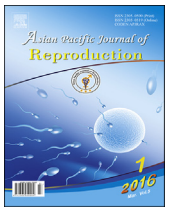

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# Different periods of intrauterine exposure to electromagnetic field: Influence on female rats' fertility, prenatal and postnatal development

Ali S.H. Alchalabi[1*], Erkihun Aklilu[1], Abd Rahman Aziz[1], F. Malek[2], S.H. Ronald[2], Mohd Azam Khan[1]

[1]*Faculty of Veterinary Medicine, UMK City Campus, Pengkalan Chepa, Locked Bag36, 16100 Kota Bharu, Kelantan Malaysia*

[2]*School of Electrical System Engineering, Universiti Malaysia Perlis (UniMAP), Pauh Putra, 02600 Arau, Perlis Malaysia*

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ABSTRACT

**Objective:** To assess the intrauterine irradiation of 1 800 MHz Global System of Mobile telecommunication on pre- and postnatal development in Sprague–Dawley rats.
**Methods:** The whole-body irradiation 1 h/day and 2 h/day was applied to the pregnant rats in three different intervals (one week, two weeks and three weeks) at SAR 0.048 W/Kg and control groups. Post-Morton findings and growth markers were monitored. Sera were collected for biochemical analysis.
**Results:** Prenatal development findings showed uterine congestion, haemorrhage, dead and reabsorbed fetuses were observed in exposure groups during 2nd and 3rd week of pregnancy unlike to control. 1st and 2nd week *in-utero* irradiation showed significant reduction with unequal and asymmetrical distribution of implantation sites and embryos in exposure groups except the control group. A number of live embryos were significantly reduced with an increasing number of dead and reabsorbed embryos in the 2 h/day of the 2nd-week exposure group in compared to control group. Malformation, haematoma, and oedematous foetuses in experimental groups were observed unlike control foetuses. A significant decrease in live foetuses and a significant decrease in body mass of foetuses at gestation day 20, unlike control group. Postnatal observations showed haematoma, congestion, short tail, malformation and growth restriction and delay in some growth markers were observed. *In-utero* irradiation for 2 and three weeks induced oxidative stress in pregnant rats.
**Conclusion:** Results suggest that long-term exposure to EMF during the pregnancy lead to chronic stress, which has detrimental effects on pre-& postnatal development and for that more studies to clarify such harmful effects are recommended.

## 1. Introduction

Potential risk of radio-frequency electromagnetic wave (RF-EMW) emitted by modern mobile phone communications technology for human environment, and health is strictly connected to contemporary approaches to assign safety limits for cell phones, wireless fidelity (Wi-Fi) as well as all electronic devices operate within microwave range. Safety limits that defined by International Commission of Non-Ionizing Radiation Protection (ICNIRP) guidelines based on the thermal effect of short-term exposure to non-ionizing radiation within microwave range on the biological system [1]. Nowadays, a huge amount of research articles of epidemiological, human, animal, cellular, mechanisms and dosimetry studies points to non-thermal effect of microwave (MW) on the biological system after chronic exposure of the environment and human population to RF-EMW (heavy use of mobile phone communication devices) [2–5]. The latest research agenda of World Health Organization (WHO) on Radiofrequency fields (RF) has prompted studies on different ages of children in addition to animal work to investigate RF exposure effect on prenatal development and behaviour and viewing the effect of RF on early life stages [6]. Electromagnetic fields (EMF) within frequency 900–1 800 MHz Global System of Mobile telecommunication (GSM) and 2 450 MHz of Wi-Fi signals have increased public concern as to health effect with exclusive attention to the

*Corresponding author: Ali S. H. Alchalabi, Faculty of Veterinary Medicine, UMK City Campus, Pengkalan Chepa, Locked Bag36, 16100 Kota Bharu, Kelantan, Malaysia.
Tel: +60 112947731
E-mail: alisaeedchalaby@yahoo.com
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adolescent population. ICNIRP and Institute of Electrical and Electronics Engineers (IEEE) do not involve pregnant women and their babies in their safety limit levels of RF-EMW exposure, although they involve workers and general public in their limit levels of RF-EMW [1,7]. WHO recently reported that "the accumulated evidence did not establish the existence of adverse short or long-term health effects from the signals produced by base stations and local wireless networks" and the "mechanistic understanding would address the possibility that children may react to RF more effectively than adults" [8]. In the last 10 years, several articles have been published showing the effect of radio-frequency radiation (RFR) intrauterine exposure on dams and their newborns. These studies investigated the effect of RFR on oxidative stress, DNA damage, teratogenicity, neurodevelopment, embryogenesis and behaviour in laboratory animals and avian species [5], [9–12]. They suggest that the RFR & GSM-like signals had adverse effects on pregnant dams as well as their newborns and had detrimental effects on avian embryos after exposure either to mobile phone or signal generator within frequency of 850 MHz–1 800 MHz. On the other hand, a few reported contradictory results regarding the effect of intrauterine Wi-Fi exposure of pregnant rats and postnatal development. Teratology and development studies have not detected any noxious effects of exposure to mobile phone-related RF fields at exposure levels below standard levels [13]. Contradictory study in Turkey found growth restriction and delayed puberty in female Wister rats due to intrauterine and early life stage exposure to Wi-Fi signal [2]. Long term "heavy use" exposure to mobile phone communications technology either via mobile phones or via mobile phone base stations radiation which is the main and important sources of RFR in our environment during different stages of pregnancy should be considered as a serious problem that needs to be addressed adequately. The lack of solid and unwavering conclusive study that establishes the deleterious effect of electromagnetic radiations from mobile phones and base stations on pregnant women, and newborns justifies the need for further and extensive studies in this regard. Therefore, this study was designed to investigate the bio-effects of 1 800 MHz GSM-like RF-EMF of mobile phone on different periods of pregnancy on female rats' fertility, prenatal and postnatal development of pups.

## 2. Material and methods

### 2.1. Animals and study design

The study was approved by the scientific committee of Faculty Veterinary Medicine of University Malaysia Kelantan (UMK) and was conducted in accordance with the UMK guidelines for animal experiments (FPV-PGSC-2014). Sprague–Dawley rats were bred at room temperature (24 ± 10) °C and humidity of (60 ± 10)% (relative humidity) with light/dark cycle 12–12 h in the laboratory animal research unit of Faculty of Veterinary Medicine, (UMK), tap water and standard rat pellet were provided *ad libitum*. For intrauterine exposure in different periods, gestation period was divided into three intervals 1st week, 2nd week and 3rd week.

Sixty virgin female rats (in each interval), 12 weeks of age at the start of experiment were used. Females were mated with unexposed adult fertile male rats of the same strain in a 1:2 ratio (♂:♀) for a maximum of 15 nights. During the cohabitation period, vaginal swab samples were examined microscopically every morning for the presence of sperm. The day of finding a copulatory plug or sperm was considered as gestation day 0. All females were observed daily for mortality and for physical signs from initiation of exposure. Animals were kept in Plexiglas cages. Especially designed exposure Plexiglas box (60 cm × 40 cm × 20 cm) was used during the exposure time because Plexiglas is non-conductive material that is not affected by RF-EMR. The protocol of RF-EMR exposure was done as follows:

a) 1st-week interval exposure was started from Gestation Day (GD) 0 until GD 7.
b) 2nd-week interval exposure was started from GD 0 until GD 14.
c) 3rd-week interval exposure was started from GD 0 until GD20 one day before delivery.

Each experimental interval was composed of:

1) Control group (n = 20).
2) 1 h/day exposure group (n = 20).
3) 2 h/day exposure group (n = 20).

At the end of each interval of the experiment, the experimental animals were divided into two subgroups; A and B. Female fertility and prenatal development were evaluated in each interval of subgroup's A females, whereas postnatal development was evaluated in pups of subgroup's B females.

### 2.2. Radiofrequency electromagnetic radiation setup

Pregnant dams were randomly distributed into nine groups (three groups/interval and 20 animals/group) SAR level 0.048 W/Kg was calculated using the equation,

$$SAR = (\sigma/P)/E^2$$

Where (E) is the magnitude of electric field 38.63 v/m, (σ) is the conductivity 1.34 s/m and (ρ) is the mass density of the tissue-equivalent media 1090 kg/m$^3$ [14]. Whole-body radiofrequency radiation (RFR) exposures were done. (Figure 1).

GSM-like signals at a frequency of 1 800 MHz were provided by a signal generator (Agilent Technologies E8267D, 250 KHz–20 GHz PSG Vector Signal Generator) with an integrated pulse modulation unit and horn antenna (A-INFOMW Standard Gain Horn Antenna 1.7–2.6 GHz WR430, China) in an exposure room. The signals were amplitude-modulated by rectangular pulses with a repetition frequency of 217 Hz and a duty cycle of 1:8 (pulse width 0.576 msec), corresponding to the dominant modulation component of the GSM. The RFR generator provided 20 dBm (0.1 W) powers during the exposure period [15–17].

### 2.3. Maternal observations

#### 2.3.1. Body weights

All animals were weighed individually on time zero (one day before starting the experiment) and at the end each interval of an experiment by using a balance with 0.01 g sensitivity.

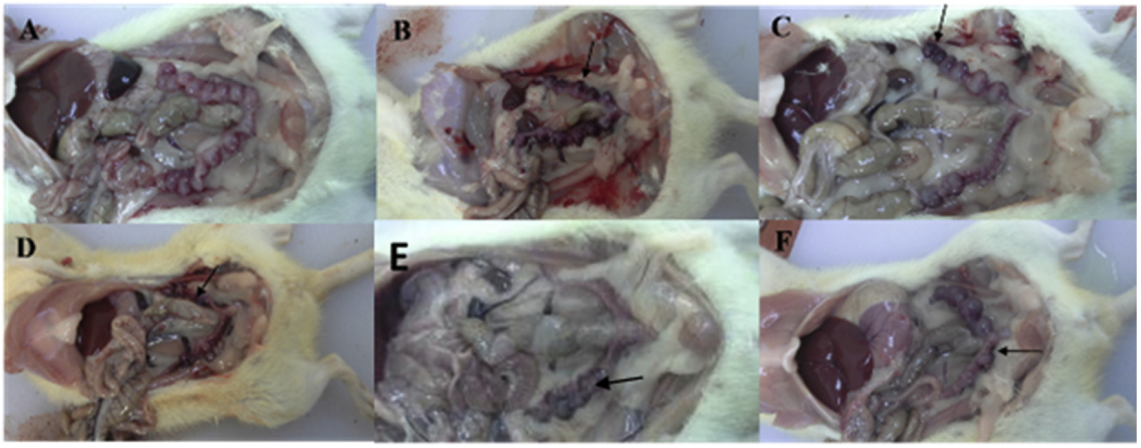


**Figure 1.** Interval 2nd week of pregnancy (A) control female shows normal uterus with normal distribution of embryos in both uterine horns, (B, C) females' uterine horns of both exposure groups show congestion, haemorrhage, dead and reabsorbed embryos and were indicated by arrows, (D, E and F) females' uterine horns of both exposure groups show abnormal distribution and decrease in number of embryos.

### 2.3.2. Clinical observation

Daily clinical examination was performed on females during experimental durations for clinical signs: abortion, mucous membrane status, behaviour and nervous signs.

### 2.4. Fertility and prenatal development

At the end of an exposure period of each interval, animals in subgroups A anesthetized by chloroform and all females were euthanized on GD7, 14 and 20, cesarean section were done. The uterus was removed, opened and pregnancy status recorded. During the 1st-week interval, the uterus of each dam was examined to determine the number of implantation sites and number of blastocysts in each uterine horn. During 2nd week interval, the uterine horns were examined to determine (1) the number of live or dead fetuses per uterine horn, (2) the number of early and late resorption sites on each uterine horn. While in duration, 3rd week of pregnancy at GD 20 total number of fetuses, number of alive, dead fetuses and fetuses were weighed individually. Macroscopic abnormalities were checked carefully.

### 2.5. Postnatal development

Females of subgroup B after finishing RF-EMR exposure for all three intervals of pregnancy were observed carefully until natural delivery happened. The pregnant females were separated individually in monitoring cages from delivery day until 30 days.

### 2.5.1. Delivery data

Each dam was examined after 24th hour of natural delivery to count the total numbers of pups/litter/group/interval, live, dead and with anomalies.

### 2.5.2. Body weight of pups

Pups/litter/group/intervals were weighed on 24 h after delivery, D7, D14, D21, and D28 individually (30 pups/group were taken for weekly weight gain).

### 2.5.3. Normal physiological development

Following a general examination, the number of pups/litter/group/interval monitoring for the following physiological development. 30 pups/group/interval were checked carefully and individually for following parameters: Ear appearance, hair appearance, teeth appearance, eye opening and weaning day as an indicator for normal growth.

### 2.6. Biochemical analysis

A rapid method for measuring malondialdehyde (MDA) as a bio-marker of lipid peroxidation in plasma samples was done, which based on the thiobarbituric acid (TBA) test by use of spectrophotometric quantification of pink MDA-TBA complex formed by reaction of MDA with two molecules of TBA. Lipid Peroxidation (MDA) Assay Kits (ab118970, abcam) were used according to the manufacturer's instructions. Briefly 10 μL plasma with 500 μL of 42 mM $H_2SO_4$ in a micro centrifuge tube was added. Add 125 μL of phosphotungstic acid solution and mix by vortexing. Incubation at room temperature for 5 min, after that centrifuge for 3 min at 13000 × *g*. The pellets were collected and resuspended on ice with 100 μL $ddH_2O$ [with 2 μL BHT (100×)]. The final volume was adjusted to 200 μL with $ddH_2O$, and then 600 μL of TBA solution into each tube containing standard and sample was added. Incubation at 95 °C for 60 min. Following reaction with TBA, MDA was directly quantified using spectrophotometer at wave length 532 nm [18].

Glutathione peroxidase (GSH-PX) concentration in serum was measured by using rat glutathione peroxidase Elisa kit (CUSA-BIORat GSH-PX ELISA Kit, CSB-E12146r, Wuhan University

**Table 1**
Effect of RF-EMR on dams' body weight during a different period of pregnancy of experiment (Table 1).

| Interval Groups | 1st Week | 2nd Week | 3rd Week |
|---|---|---|---|
| Control | 257.600 ± 8.128 | 288.700 ± 6.298 | 323.400 ± 6.172 |
| 1 h/day exposure | 272.200 ± 8.594 | 275.600 ± 8.466 | 335.200 ± 3.072 |
| 2 h/day exposure | 257.100 ± 7.004 | 276.500 ± 11.590 | 330.700 ± 3.343 |
| *P* value | [b]0.204<br>[c]0.065 | [b]0.315<br>[c]0.345 | [b]0.070<br>[c]0.253 |

Values are mean ± S.E. [b]Comparison between 1 h/day exposure group and the control group, [c]Comparison between 2 h/day exposure group and the control group, respectively.

Science, Wuhan, Hubei province 430223, P.R.China). According to the manufacturer's instructions, estimation was done.

Melatonin (MT) concentration in serum was estimated by using rat melatonin Elisa kit (CUSABIO MelatoninELISA Kit, CSB-E13433r, Wuhan University Science, Wuhan, Hubei province 430223, P.R.China). Estimation was done according to the manufacturer's instructions. Both GSH-PX and MT measurement were done as following procedure. Briefly, Reagents, samples and standard were prepared and 100 µl of samples and standard was added to wells and incubated 2 h at 37 °C. Each well was removed from the contents and 100 µl of biotin-antibody was added and incubated for 1 h at 37 °C. Washing three times with washing solution, 100 µl HRP-avidin was added to each well and incubated for 1 h at 37 °C. After that each well was washed 5 times by washing solution and 90 µl TMB substrate was added in each well, incubated for 20 min and finally 50 µl stop solution was added to each well. Spectrophotometer was used at wave length at 450 nm for reading the assay plate.

### 2.7. Statistical analysis

All results were expressed as mean ± standard error (mean ± S.E.). Data were evaluated by using the statistical SPSS program v.19 software (SPSS In. Chicago, IL., USA). ONE and TWO-way ANOVA and LSD test was used to evaluate the significance between groups and *P* values of less than 0.05 were considered as significant.

## 3. Results

### 3.1. Dam's body weight

ANOVA test for dams' body weight revealed that there were no significant differences in mean body weight of the dams in experimental groups during 1st, 2nd and 3rd-week intervals.

### 3.2. Clinical observations

Clinical examination showed rough skin was observed in some females in 2 h/day exposure group of an interval 3rd-week experiment. Abortion and bleeding were observed in one female of 1 h/day exposure group of the interval 3rd-week experiment. While no abnormal clinical signs were observed in other females in experimental groups of intervals 1st week and 2nd week. No death was noted in all intervals and groups of experiment. Post Morton (PM) findings showed uterine congestion, haemorrhage,

**Table 2**
Effect of RF-EMR on prenatal development during different periods of pregnancy.

| Parameters Groups | Duration of exposure | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | One week | | Two weeks | | | Three weeks | | | |
| | No. of implantation sites | No. of blastocysts | No. of live embryos | No. of dead embryos | No. of reabsorbed embryos | Total no. of foetuses | No. of live foetuses | No. of dead foetuses | Weight of foetuses at GD 20 (g) |
| Control | 10.80 ± 0.89 | 10.30 ± 0.87 | 10.20 ± 0.69 | 0.80 ± 0.32 | 0.40 ± 0.22 | 11.30 ± 0.53 | 10.60 ± 0.61 | 0.70 ± 0.33 | 5.670 ± 0.06 |
| 1 h/day exposure | 9.00 ± 0.55 | 8.40 ± 0.81 | 7.60 ± 1.36 | 1.90 ± 0.5 | 1.30 ± 0.33 | 7.80[c] ± 0.62 | 7.00[c] ± 0.57 | 0.80 ± 0.24 | 4.424[c] ± 0.09 |
| 2 h/day exposure | 8.50[a] ± 0.58 | 7.90[a] ± 0.67 | 7.20[a] ± 0.9 | 2.30[a] ± 0.51 | 1.60[a] ± 0.37 | 8.30[c] ± 0.55 | 7.50[c] ± 0.54 | 0.80 ± 0.24 | 4.900[c] ± 0.09 |
| *P* value | 0.078<br>0.027 | 0.101<br>0.041 | 0.085<br>0.049 | 0.101<br>0.028 | 0.054<br>0.012 | 0.001<br>0001 | 0.001<br>0.001 | 0.803<br>0.803 | 0.001<br>0.001 |

Values are mean ± S.E. [a]$P < 0.05$, [c]$P < 0.001$ compared to the control group.

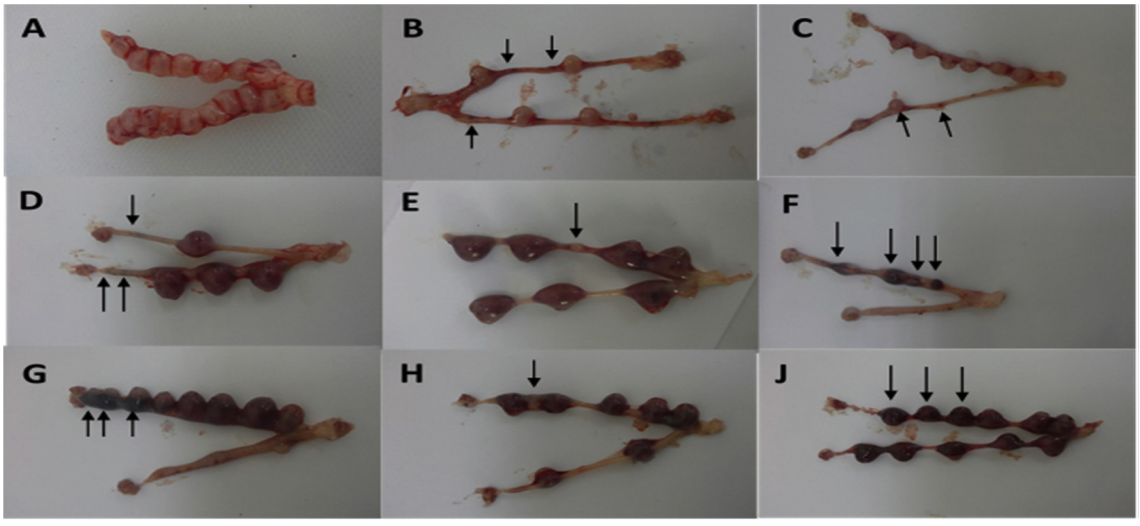


**Figure 2.** Interval 2nd week of pregnancy. (A) Control normal uterus with normal distribution of embryos in both uterine horns, (B, C, D) early embryonic death was observed in RF-EMR exposure groups and indicated by arrows, (E, H) reabsorbed embryos were recorded in 1 h/day exposure group, (F, G) early embryonic death was observed in RF-EMR exposure groups, (G, H, J) moderate to severe congestion was observed in uterine horns in all exposure groups. Unequal and asymmetrical embryonic distributions were very clear in (B, C, D, E, F, G, H, and J) unlike to (A) control uterine horns.

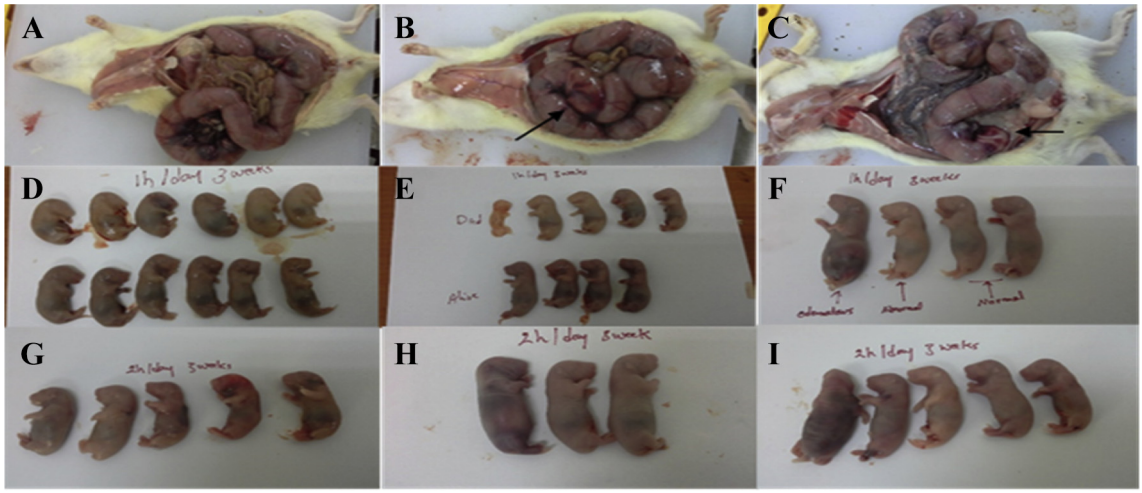


**Figure 3.** Interval 3rd week of pregnancy. (A) Control normal uterus with normal foetuses, (B, C) 1 h/day and 2 h/day exposed rats' uterus showed haemorrhage and congestion indicated by an arrow. (D, E, F) GD 20 foetuses showed haematoma, dead and oedematous foetuses in 1 h/day exposed rats. (G, H, I) Sever haematoma with big size oedematous foetuses were observed in GD 20 of pregnancy in 2 h/day 2 h/day exposure group.

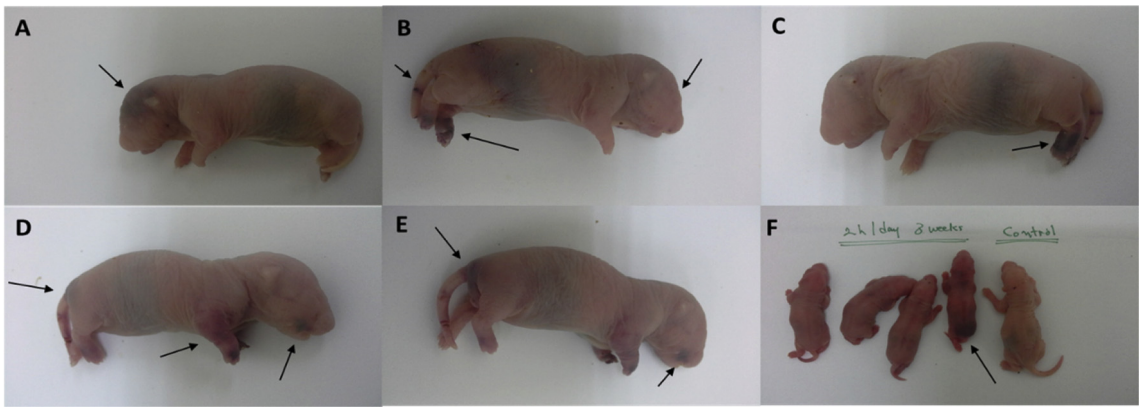


**Figure 4.** Intrauterine exposure to RF-EMR showed different hazardous effects. (A) Head haematoma with head deformity was observed in pup after exposure to 1 h/day for three weeks. (B) Short tail, head deformity and haematoma on left foot and tail were observed in 1 h/day exposure for three weeks. (C) left foot haematoma in pup after exposure to 1 h/day irradiation. (D, E) haematoma on the base of the tail, on the lower jaw and skin redness with haematoma on the right arm after 2 h/day intrauterine irradiation for three weeks. (F) congested pups with haematoma on the back and base of the tail on pup in the 2 h/day exposure group.

dead and reabsorbed fetuses were observed in both exposure groups during 2nd and 3rd intervals of pregnancy, while no PM findings were observed in females of control groups (Figure 1).

### *3.3. Fertility and prenatal development (laparotomy data) for subgroups A female*

#### *3.3.1. One-week exposure duration (GD 0–GD 7)*

Number of implantation sites and number of blastocysts in the 2 h/day exposure group were affected markedly with unequal and asymmetrical distribution of implantation sites in both uterine horns unlike the control group (Table 2) (Figure 2).

#### *3.3.2. Two weeks exposure duration (GD 0–GD 14)*

Two-week exposure to RF-EMR showed that the mean number of live embryos was significantly reduced, while a number of dead and reabsorbed embryos were increased significantly in the 2 h/day exposure group in comparison with a control group (Table 2). 1 h/day exposure to RF-EMR has no significant effect on a number of alive, dead and reabsorbed embryos despite of there were some alterations in their values. Asymmetrical distribution of embryos was also observed in both uterine horns in both RF-EMR exposure groups except the control group (Figure 2).

#### *3.3.3. Three-week exposure duration (GD 0–GD 20)*

The uterine horns were affected significantly and exhibited some pathological signs such as congestion and haemorrhage in irradiated females of both exposure groups in comparison with a

**Table 3**
Effect of one-week intrauterine RF-EMR exposure on off-spring examination.

| Groups | Total no. of pups | No. of live pups | No. of dead pups | No. of anomalies |
|---|---|---|---|---|
| Control | 10.20 ± 0.55 | 10.10 ± 0.54 | 0.10 ± 0.10 | 0.30 ± 0.15 |
| 1 h/day exposure | 9.70 ± 0.59 | 9.50 ± 0.58 | 0.20 ± 0.13 | 0.30 ± 0.21 |
| 2 h/day exposure | 9.60 ± 0.61 | 9.50 ± 0.58 | 0.10 ± 0.10 | 0.20 ± 0.13 |
| *P* value | [b]0.554<br>[c]0.479 | [b]0.464<br>[c]0.464 | [b]0.534<br>[c]1.000 | [b]1.000<br>[c]0.681 |

Values are mean ± S.E. [b]Comparison between 1 h/day exposure group and the control group, [c]Comparison between 2 h/day exposure group and the control group.

**Table 4**
Effect of two weeks intrauterine RF-EMR exposure on off-spring examination.

| Groups | Total no. of pups | No. of live pups | No. of dead pups | No. of anomalies |
|---|---|---|---|---|
| Control | 10.50 ± 0.58 | 10.40 ± 0.60 | 0.10 ± 0.10 | 0.10 ± 0.10 |
| 1 h/day exposure | 8.30 ± 0.59 | 7.90 ± 0.52 | 0.40 ± 0.22 | 0.40 ± 0.22 |
| 2 h/day exposure | 6.40 ± 0.60 | 6.00 ± 0.57 | 0.40 ± 0.22 | 0.50 ± 0.16 |
| *P* value | [b]0.014 | [b]0.004 | [b]0.273 | [b]0.223 |
| | [c]0.001 | [c]0.001 | [c]0.273 | [c]0.108 |

Values are mean ± S.E. [b]Comparison between 1 h/day exposure group and the control group, [c]Comparison between 2 h/day exposure group and the control group.

**Table 5**
Effect of three weeks RF-EMR intrauterine exposure on off-spring examination.

| Parameters | Total no. of pups | No. of live pups | No. of dead pups | No. of anomalies |
|---|---|---|---|---|
| Control | 11.30 ± 0.49 | 11.10 ± 0.48 | 0.20 ± 0.13 | 0.20 ± 0.13 |
| 1 h/day exposure | 7.80 ± 0.74 | 7.50 ± 0.67 | 0.30 ± 0.15 | 0.60 ± 0.22 |
| 2 h/day exposure | 7.50 ± 0.68 | 7.20 ± 0.64 | 0.30 ± 0.15 | 0.50 ± 0.22 |
| *P* value | [b]0.001 | [b]0.001 | [b]0.633 | [b]0.163 |
| | [c]0.001 | [c]0.001 | [c]0.633 | [c]0.292 |

Values are mean ± S.E. [b]Comparison between 1 h/day exposure group and the control group, [c]Comparison between 2 h/day exposure group and the control group.

control group. Intrauterine exposure for three weeks affected negatively the prenatal development of foetuses represented by malformation, haematoma in different parts of the body, increase in skin thickness and oedematous foetuses (Figure 3). Intrauterine irradiation by 1 800 MHz GSM-like signals in 1 h/day and 2 h/day groups exhibited a highly significant decrease in a mean total number of foetuses and number of live foetuses in comparison with non-irradiated dams in a control group, while a number of dead foetuses remain consistent. Body mass of foetuses at GD20 was affected significantly in both irradiated groups (Table 2).

## 4. Postnatal development (delivery data) for subgroup's B pregnant females

Exposure to RF-EMR in-utero during different intervals of pregnancy lead to different hazardous effects on live births and

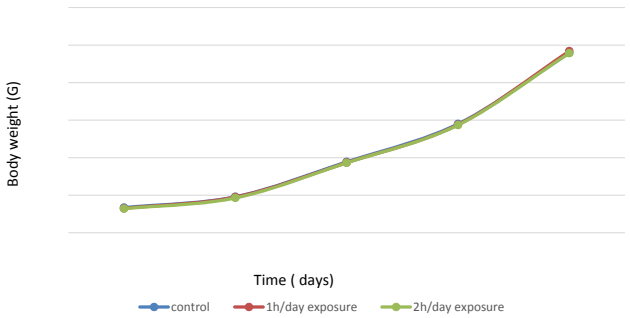


**Figure 5.** Effect of RF-EMR intrauterine exposure for one week of pregnancy on weekly body weight gain of pups.

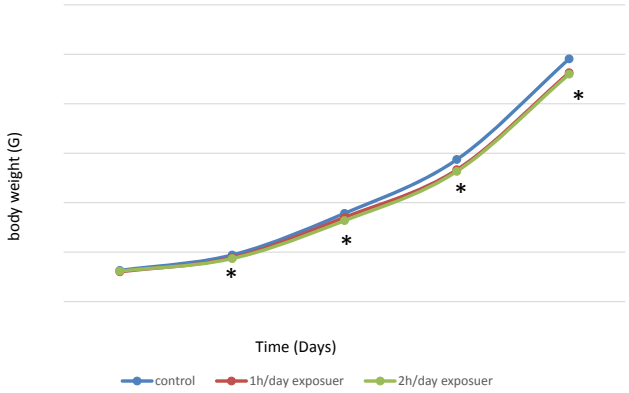


**Figure 6.** Effect of RF-EMR intrauterine exposure for two week of pregnancy on weekly body weight gain of pups.

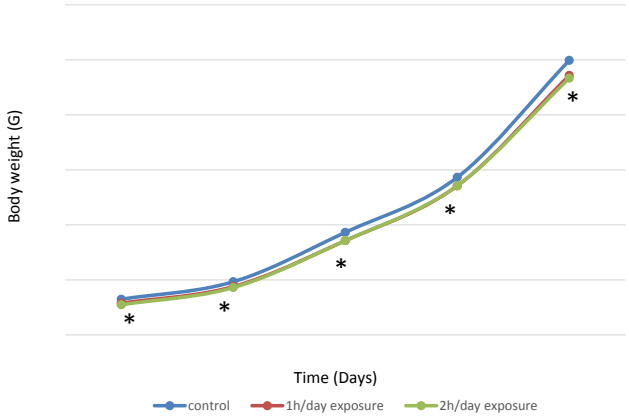


**Figure 7.** Effect of RF-EMR intrauterine exposure for 20 days of pregnancy on weekly body weight gain of pups.

these effects include haematoma, rough skin, skin redness, congestion, short tail and malformation and manifestations of these signs depend on duration of intrauterine exposure (Figure 4).

### 4.1. Delivery data

Mean total number of newly born pups, number of live and dead pups in addition to a number of pups with anomalies after in-utero irradiation with 1 800 MHz GSM-like RF-EMR during the 1st week of pregnancy were similar in all three groups. After two-week intrauterine exposure the delivery data exhibited a

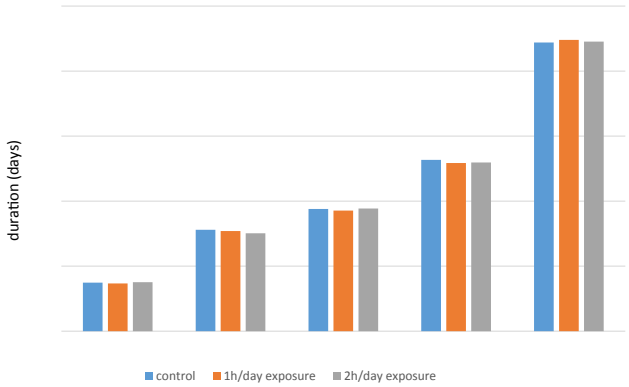


**Figure 8.** Effect of one week RF-EMR intrauterine exposure on postnatal physiological development.

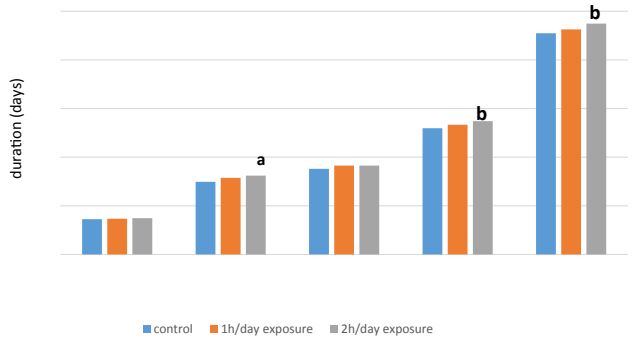


**Figure 9.** Effect of two weeks RF-EMR intrauterine exposure on postnatal physiological development.

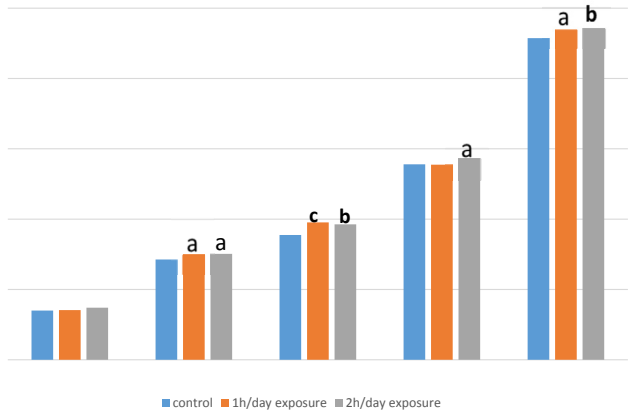


**Figure 10.** Effect of three weeks RF-EMR intrauterine exposure on postnatal physiological development.

reduction in a total number of pups and number of live pups unlike control group pups. While dead and abnormal pups number similar to control pups. Total number of pups and live pups in exposure groups after three weeks of exposure were significantly reduced in comparison with control group and the number of dead and normal pups statistically remain normal (Tables 3–5).

### 4.2. Pups weekly body weight gain

Mean body weight was taken for 30 pups/group/week and measured weekly beginning from 24 h after normal delivery until 28 days of age (24 h, D7, D14, D21 and D28). Pup's body weight after intrauterine exposure to RF-EMR during 1st week of pregnancy showed no effect and were similar in all experimental groups (Figure 5).

Body weights of pups aged 24 h from *in-utero* irradiated dams for two weeks did not show significant difference compared with the control pups ($P = 0.551$, $P = 0.335$ 1 h/day and 2 h/day exposure respectively). While the weekly body weight gains from D 7–D 28 was significantly lower than control groups ($P = 0.001$). Highly significant reduction in weekly body weight gain in new-born pups until D 28 ($P = 0.001$) was observed in pups from in-utero irradiated dams for 20 days in both exposed groups compared with the control pups (Figures 6 and 7).

### 4.3. Normal physiological development

Postnatal normal physiological development was checked carefully and individually for 30 pups/group/interval for monitoring appearance of ear, hair, teeth, eye-opening and determining of weaning day. There were no remarkable differences among experimental groups and control group for postnatal physiological development after intrauterine exposure during 1st week of pregnancy (Figure 8). No difference was observed in ear appearance development after two-week of in-utero irradiation, 2 h/day *in-utero* irradiation hair appearance, eye opening development and weaning day took longer time than control pups, while after 1 h/day irradiation, the postnatal physiological development remains consistent (Figure 9). Three weeks of *in-utero* irradiation effect on postnatal physiological development negatively by increased the days required for normal hair appearance and induced delay in teeth eruption in both irradiated groups. Eye opening took longer time to open normally than a control group after 2 h/day exposure group and the weaning period showed an increase in length of weaning period in both *in-utero* irradiation groups in comparison to control group (Figure 10).

### 4.4. Effect of RF-EMR 1 800 MHz GSM-like frequency on oxidant and antioxidant status in rats during different periods of pregnancy

In this study, it was investigated whether the 1 800 MHz GSM-like frequency exposure induced oxidative stress alters the antioxidant enzyme and hormone activity for scavenging free radicals. Antioxidant activities were determined biochemically and by using commercial ELISA kits. GD 0–GD 7 periods of

**Table 6**

Effect of 1 800 MHz GSM-like RF-EMF on melatonin level, GSH-PX activity and MDA level during the different period of pregnancy period. Effect of 1 800 MHz GSM-like RF-EMF on melatonin level, GSH-PX activity and MDA level during the different period of pregnancy period.

| Groups | GD0–GD7 | | | GD0–GD14 | | | GD0–GD20 | | |
|---|---|---|---|---|---|---|---|---|---|
| | MT (pg/mL) | GSH-PX (mIU/mL) | MDA (nmol/mL) | MT (pg/mL) | GSH-PX (mIU/mL) | MDA (nmol/mL) | MT (pg/mL) | GSH-PX (mIU/mL) | MDA (nmol/mL) |
| Control | 42.966 ± 1.50 | 64.869 ± 3.61 | 1.561 ± 0.12 | 42.221 ± 0.04 | 74.512 ± 4.71 | 1.515 ± 0.11 | 41.0132 ± 1.79 | 71.253 ± 2.34 | 1.523 ± 0.09 |
| 1 h/day Exposure | 42.212 ± 1.16 | 58.614 ± 2.45 | 1.627 ± 0.17 | 40.512 ± 0.79 | 67.411 ± 0.99 | 1.899[a] ± 0.11 | 38.561 ± 0.48 | 66.563[a] ± 0.99 | 1.973[a] ± 0.17 |
| 2 h/day Exposure | 40.446 ± 0.77 | 57.569 ± 0.77 | 1.823 ± 0.06 | 40.108 ± 0.90 | 64.511[a] ± 1.34 | 1.901[a] ± 0.12 | 37.447[a] ± 0.81 | 63.518[c] ± 0.84 | 2.093[c] ± 0.10 |
| *P* value | 0.657 | 0.096 | 0.723 | 0.201 | 0.093 | 0.028 | 0.133 | 0.042 | 0.021 |
| | 0.145 | 0.054 | 0.292 | 0.117 | 0.021 | 0.027 | 0.035 | 0.002 | 0.045 |

Values are mean ± S.E. [a]P < 0.05, [c]P < 0.005 are accepted to be statistically significant, p value was comparison between the control group and two treatment groups respectively.

intrauterine exposure to RF-EMR showed that the oxidant and antioxidant activity in all experimental groups remain consistent. After 2 h/day intrauterine exposure from GD 0-GD 14 GSH-PX activity was significantly lower than the observation in the control group. While 1 h/day exposure showed no effect. MDA level was higher significantly in both exposure groups in comparison to control group. GD 0–GD 20 intervals showed a significant decrease in MT level after 2 h/day intrauterine exposure and significant decrease in GSH-PX activity in both exposure groups in comparison to control group. Both intrauterine exposure groups showed a significant increase in MDA level compare to control group. The results are shown in (Table 6).

## 5. Discussion

Fetal growth is one of the most sensitive stages of growth to electromagnetic radiation because developing tissues and organogenesis is more sensitive to harmful agents than those of adults. There are no consistent results, until now, about the effect of RF-EMR on prenatal and postnatal development due to a difference in experimental design, including SAR, frequencies, duration of exposure and short or long-term exposure although all the frequencies used within the microwave range. Our study differs from previous studies in that we investigated the biological effects of frequency 1 800 MHz GSM RF-EMR through different periods of pregnancy and its effect on the growth and development of embryos during pregnancy and after pregnancy. We elucidated that 1 800 MHz GSM radiation during pregnancy caused some detrimental effects on prenatal development as represented by significant unequal and asymmetrical distribution of implantation sites in both uterine horns of exposed groups except the control group, reduction in foetal weight with some anomalies as well as induction of oxidative stress in pregnant animals, decrease in weekly body weight gain and some negative effect on functional and physiological development. Previous studies have addressed various aspects of the impact of 1 800 MHz frequency during pregnancy. For instance, Tomruk *et al.* found that exposing rabbits within middle stage of pregnancy for 15 min/day for seven days to 1 800 MHz GSM-like signals lead to oxidative destruction in hepatic tissue as a result of production of free radicals in pregnant animal [19]. Another study performed by other Turkish team indicated that 1 800 MHz GSM-like signal's exposure of non-pregnant and pregnant rabbits for seven days (15 min/day) resulted in producing free radicals who induce lipid peroxidation and oxidative DNA damage [15], Foetal exposure to 800–1 900 MHz-rated radiofrequency radiation from cellular phones lead to changes in behavioural and neurophysiological parameters (impaired memory, hyperactive and decreased anxiety) that persist into adulthood when mice exposed during pregnancy [9]. Our findings were similar with other recent studies on in-utero exposure effect(s) of Wi-Fi frequency 2.45 GHz on pregnant animals and their infants (pre & postnatal developments). In one of such study, mice exposed to 2.45 GHz EMF for 2 h/day for 30 days as (pre-mating period 22 day, mating period 5 days and post-mating 3 days) exhibited asymmetrical and unequal implantation sites in uterine horns in exposed groups compared to control and induced DNA break in brain cells [20]. In another study mice exposed to 2.45 GHz microwave in-utero irradiation continuous wave 2 h/day for 45 days at SAR 0.023 023 W/kg as (pre-mating period 20 days, 5 days during mating and 20 days post-mating periods), this study showed that the MW irradiation induce oxidative stress which suppresses implantation (unequal/asymmetrical distribution of embryos were observed in the uterine horns) and lead to embryonic deformity in case pregnancy continues and lead to DNA strand break in brain cells [21]. Also our findings is in agreement with [4] who found that pregnant rats exposed to 2 450 MHz EMF resulted in postnatal growth restriction and delayed puberty in female rats due to induce oxidative stress in brain and ovarian tissues. Our results can be interpreted that the susceptibility for subfertility and prenatal growth impairment due to early embryonic death and resorption of embryo during second week of pregnancy was as a consequence of intrauterine irradiation inducing oxidative stress which play important role in implantation and embryonic growth via inhibition of specific heat shock proteins (Hsp) such as Hsp70 and Hsp105. Hsp70 plays a critical role in fertilization and early embryonic development in mammalian. Furthermore, Hsp may also serve as a protective role in embryo development since inhibition of Hsp70 caused a reduction in blastocyst development that may be mediated by high rate of apoptosis [22], while temporary and spatial changes in Hsp105 expression in pregnant rat uterus play an important physiological role in regulating embryo implantation [23]. The finding that RF-EMR influences prenatal development reflects the influence of many Hsps should be investigated further more. Oxidative stress is caused by the excessive production of free radicals or from unbalance of oxidant/antioxidant system. Our results data clearly show that RF-EMR (1 800 MHz) at low SAR leads to decrease in GSH-PX activity & MT levels as antioxidant enzyme and free radical scavenger as well as increase in MDA level, a biomarker for lipid peroxidation and tissue damage due to extensive production of peroxides and free radicals that cause toxic effect and damages all cell components for instance protein, lipids and DNA.

Reactive oxygen species (ROS) play an important role in the physiological reproductive functions such as maturation, ovarian steroidogenesis, corpu luteal function, fertilization, embryo development and pregnancy [24]. Despite the huge reports indicating, non-ionizing radiation induced oxidative stress and its effect on implantation, prenatal development and pregnancy, the precise mechanism of action of RF-EMR in inducing early embryo loss, malformation and impair pregnancy still unclear. More detailed studies should be conducted to determine the specific free radicals and Hsps contribution to prenatal development in different stages of embryogenesis. Postnatal development results showed a decrease in weekly body weight gain and delay in some functional and physiological development such as teeth & hair appearance, eye-opening and prolonged weaning period. This may be as a result of interference between the impact of oxidative stress and growth factors such as an insulin growth factors-I IGF-I in the body. Our result is consistent with that of Dundar *et al.* which showed 50 Hz exposure resulted in growth restriction, delay puberty and reduced IGF-I level in female rats and probably associated with direct toxic effect of electric field on target organs [25]. IGF-I have a very important role in foetal and postnatal development. The variation in IGF-I levels associated with EMF exposure have been reported in few studies. Sangun *et al.* found that female rats exposed to 2.45 GHz during pregnancy, and postnatal development showed a decrease IGF-I level in the postnatal group compared to control animals as a result of induction of oxidative stress [4]. Picinato *et al.* found that MT via

activation of MT 1 receptor regulates growth and differentiation of pancreatic islets by activating signalling pathway of IGF-I and insulin receptors [26]. In 2009, Oner conducted a study to investigate whether or not MT has a role in the prevention of carbontetrachlorid induced hepatotoxicity in rats by IGF expression. They found that MT secreted from pineal gland increases IGF-I was releasing and prevents hepatic damage [27]. Postnatal growth restriction in our experiment may be interpreted as due to decrease in MT level in experiment of three-week exposure to irradiation, which has the effect on releasing of IGF-I that have an important role in postnatal growth. Our results are in contrast with other recent studies. For instance, mice were intrauterine exposed 2 h/day for 14 days, starting after five days of mating to Wi-Fi signal at 2.45 GHz, SAR 4 W/kg and the result revealed no effect on pregnancy outcome due to prenatal exposure to Wi-Fi signals [28]. In another study, rats were in-utero exposed to 2.45 GHz Wi-Fi signals (2 h/day for 18 days at SAR 0.08, 0.4 and 4 W/kg) to investigate the pre- and postnatal effect of Wi-Fi signals and no abnormalities were noted in pregnant rats, no signs of toxicity in the pre-and postnatal development of pups [13]. In a study conducted in France rats were exposed to 2.45 GHz Wi-Fi signals as whole-body exposure of free-moving rats for 1 h/day for 3 weeks (male) two weeks (female) during sexual maturation, and the study suggested that Wi-Fi signals have no effect on male and female fertility at" WB SAR values up to the ICNIRP critical level" [29]. The current study, exposure to RF-EMR 1 800 MHz during different periods of pregnancy resulted in different effects on pregnancy outcome and delayed in normal, functional and physiological postnatal development in pups from intrauterine irradiated dams. The frequency and SAR were used in our experiment were within safety limits of ICNIRP & IEEE guidelines. According to experiment results, we suggest that long-term exposure to EMF within microwave frequency during a pregnancy lead to chronic stress which in turn may cause some detrimental effects on pre- and postnatal development via different pathways, for that an additional studies need to be conducted to clarify the actual pathways that involve in such harmful effects.

## Conflict of interest statement

We declare that we have no conflict of interest.

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