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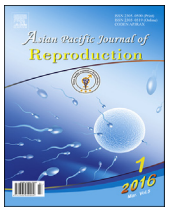

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Different periods of intrauterine exposure to electromagnetic field: Influence on female rats' fertility, prenatal and postnatal development

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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

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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.