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Original Article

Biological effects of cell-phone radiofrequency waves exposure on fertilization in mice; an *in vivo* and *in vitro* study

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ABSTRACT

Increasing use of cell-phone is one of the most important risk factors for population health. We designed an experimental study aimed at evaluating the effects of cell-phone radiofrequency (RF) waves exposure on fertilization in mice. Two hundred male and female NMRI-mice were used. One hundred males divided in five groups (n = 20) as control and exposed groups. Those irradiated with cell-phone RF in "Standby-mode" 1, 5 and 10 h daily named groups II, III and IV; respectively. Group V irradiated with cell-phone on "Active-mode" one hour daily. After 30 days irradiation, 50 males and 50 females were kept 24 h to assess their embryos. Fifty males were scarified to evaluate both *in vitro* and *in vivo* parameters, and 50 females received PMSG & HCG for both quantitative and qualitative evaluation. Comparing groups III, IV and V with control-group showed significantly decreased in the number of two-cell embryos (p = .000); however, a significant increase was found in the number of dead embryos (p = .000). Furthermore, 5 h daily irradiation significantly decreased grade-A embryos (p = .015); while, it significantly increased grade-B, C and D embryos (p-values = 0.026, 0.007, 0.006; respectively). Moreover, comparing groups IV and V to control-group, significant increase was found in pregnancy duration (p = .005, p = .009; respectively). However, in the mentioned groups a significant decrease was seen in number of newborn mice (p = .001, p = .004; respectively). In conclusion our findings showed that the cell-phone radiation can affect development of embryos as well as the number of newborn and pregnancy duration in NMRI-mouse, which might be a significant cause of reproductive failure.

1. Introduction

Fertility is the ability to have a child and success in reproduction [1]. On the other hand, infertility is the malfunction in reproduction and the problems relating this matter is known as one of the most important issues in couples' life [1,2]. Reports have shown that approximately 35% of the infertility problems are related to men and 40% to women [3]. The most common cause of males' infertility is their inability to produce enough healthy and active sperm [3,4]. In the last few decades, the quality of sperm and its fertility power has had a significant decrease throughout human society [1,4]. This shows that the mentioned quality has been influenced by changes which are rooted in the toxic factors in the medium; such as chemical intoxication and being exposed to a variety of underlying, medical or military radiation [4]. Radio frequency (RF) waves of cell-phones and other electronic equipment, affects the biological system via thermal and non-thermal effects [5–8]. More than 50 studies, that have been investigated the RF effects on different mice, indicated increased frequencies of hypodiploid in mammalian oocytes, fertility loss, impaired spermatogenesis, and reduction of viable embryos in mice [6,8–11]. When the RF waves are absorbed in the body, they contain energy that can produce free radicals. Free radicals can break a chemical bond and become a chain of biological events including damage in the cell membrane of sexual cells [12]. Based on studies, cell-phone radiofrequency (RF) waves have negative effects on sexual

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actions [13]. Reproductive disorders have been reported in both males and females which were caused by oxidative stress, and the effect of reactive oxygen species (ROS) levels on zygotes and on the growth of the fetus has been determined [13,14]. Although ROSs produced by radiation are toxic, intracellular ROS produced in physiological conditions is regulated as essential signal molecules that regulate multiple cellular processes, the ROS spectrum produced in the short run after radiation is similar to metabolic processes [13,15]. However, there is a cellular and physiopathological distribution (single molecules and ROS clusters produced by radiation versus single molecules produced by intrinsic processes) and production time (chronic release of ROS in-production versus instantaneous production during radiation) [15,16]. Oxygen's free radical *in vitro* could affect embryonic development, clinical pregnancy rate and fertility [15]. In both *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) raising the concentration of ROS *in vitro* on the first day showed its association with reduced pregnancy rate [15]. As a result, while the damage from ROS metabolism is randomly distributed in DNA, radiation damage from DNA often occurs in clusters [16]. The purpose of this study was to investigate the effect of 900 MHz cell-phone RF waves on the quality and quantity of the NMRI-mouse embryo (from two-cell to the blastocyst resulting from IVF) as well as impact of the RF on the pregnancy duration and number of the newborn mice.

2. Materials and methods

2.1. Grouping and irradiation

In this experimental study, 200 NMRI mice (100 males and 100 females) aged 6–8 weeks and weight 20–30 g were randomly selected and were purchase from Pasture Institute (Tehran, Iran). The mice were kept in 40 similar wood cages (5 female or 5 male mice in one separate cage) in the animal house of the Shahrekord University of Medical Sciences (Shahrekord, Iran). The animal house had standard conditions of 20 ± 2 °C temperature, brightness/darkness of 12:12 h, and free access to food and water for the mice. The mice were kept one week in the animal house to adapt with the environment. The 100 male mice were randomly divided in five groups (n = 20) as: group I or control group in which the cell-phone was off, and four exposed groups. In the three exposed groups of II, III, and IV the male mice were irradiated with cell-phone RF for 1, 5 and 10 h a day; respectively, while the cell-phone was on "Standby-mode" *i.e.* the cell-phone was ON but no conversation occurred. In the 5th group (group V) the male mice were irradiated with cell-phone RF when it was on "Active Mode" (conversation occurred) one hour daily. The RF irradiation was performed for 30 days. The applied frequency of the waves was 900 MHz irradiated from a Nokia cell-phone (Nokia 1100, Finland). In case of irradiation, the distance between cell-phone and mouse was 10 cm. After that the RF irradiation was finished, the experiment was continued in four parts as follow:

2.1.1. Sperm collecting for IVF

In order to collecting sperm for IVF, fifty male mice (10 mice from control group and 10 mice from each of the four exposed group) were randomly selected. The mice were scarified by cervical dislocation on the day 31 (one day after RF irradiation was finished). In the next step, the mouse skin and peritoneum were opened, the epididymis as well as vas deferens isolated, and transferred into a Petri-dish. The Petri-dish was contained human tubal fluid (HTF) medium which previously reached equilibrium. Afterwards, epididymis and vas deferens were divided into smaller pieces and were kept for bearing capacitating 1–2 h inside a 37 °C incubator with 5% CO_2.

2.1.2. Super ovulation and oocyte collecting

For super ovulation and oocyte collecting in each of the 50 mature female mice the two following hormones were intraperitoneally injected: 10 (IU) PMSG (Sigma Co, USA) and 48 h later 10 (IU) hCG (Sigma Co, USA). Ovulation occurred 10–13 h after the hCG injection. Then, 12 h later, the skin of the injected mouse was sterilized with alcohol and killed by cervical vertebra dislocation. After that, its skin and peritoneum were removed. In the next step, the oocyte and the cells around it (*i.e.* cumulus oophorus) were collected from both sides of fallopian tube and were transferred into a Petri dish containing HTF medium. After washing the droplets of HTF, a total of 100 oocytes were obtained from female mice randomly.

2.1.3. In-vitro fertilization (IVF)

For the IVF 5 μL of the collected sperms were taken with a sampler from those having swimming up movement that were already capacitated and kept in the incubator. We added them to the drops containing 100 oocytes in a way that each ml was including 1×10^6 sperm. Then, it was kept for 5 h inside a 37 °C incubator with 5% CO_2. During this period the sperm nucleus enters the oocyte and the male and female pre-nuclei were detectable using a microscope (SMZ2, Nikon, Tokyo, Japan). About 5 h after adding the sperm, the embryos were transferred into Petri dishes containing 5 drops of KSOM medium (Millipore, Madison, WI, USA). After being washed in the 4 side drops, the embryos were transferred to the 5th drop in the middle of Petri dish. At this time the embryos were free of any impurities To evaluate the quantitative process of IVF, 24 h after the insemination, the number of two-cell (and possibly 4-cell) embryos were counted and recorded under a stereomicroscope. This continued until the embryos reached the blastocyst stage in the following days. Moreover, to assess the qualitative process the two-cell embryos were examined in terms of morphology under a stereomicroscope and according to the Bolton grading scale were grouped into four categories of grades A, B, C and D [17].

2.1.4. In-vivo assessment

In this part of the study in order to *in vivo* assessment from 40 of the remained exposed mice (group II, III, IV, V) and 10 non exposed mice (control group), one mouse was randomly selected. The mouse was kept together with one of the 50 remained females in a cage for 24 h (overnight) separately. In the next morning, to make sure of the mating, presence of plaques in the female mice's vagina, it was examined and they were recorded as positive if so. Nineteen days later, if the birth had occurred, the most important *in vivo* factors of reproduction characteristics (*i.e.* pregnancy duration and the number of newborns) were registered for each mouse.

2.2. Statistical analysis

In order to analysis the data we applied the Kolmogorov-Smirnov test, analysis of variance (ANOVA) followed by Tukey's-test. SPSS software (Version 18; SPSS Inc., Chicago, USA) was used for the statistical analysis. The p-values are two-sided at a significance level of ≤ 0.05.

3. Results

3.1. Quantitative findings of IVF

Results of our quantitative evaluation are presented in Table 1. As Table 1 shows one day after the IVF there was a significant decrease in the number of two-cell embryos for groups III, IV and V (comparing to the control group); while there was an increase

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