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Effects of Simulated Mobile Phone Electromagnetic Radiation on Fertilization and Embryo Development

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ABSTRACT

This study investigated the effects of 935-MHz electromagnetic radiation (ER) on fertilization and subsequent embryonic development in mice. Ovulating mice were irradiated at three ER intensities for 4 h/day (d) or 2 h/d for three consecutive days; the ova were then harvested for *in vitro* fertilization to observe the 6-h fertilization rate (6-FR), 72-h morula rate (72-MR), and 110-h blastula rate (110-BR). Compared with the control group, the 6-FR, 72-MR, and 110-BR were decreased in the low ER intensity group, but the differences were not significant; in the mid- and high-intensity ER groups, 72-MR and 110-BR in the 4 h/d and 2 h/d subgroups were decreased, showing significant differences compared with the control group. Moreover, the comparison between 4 h/d and 2 h/d subgroups showed significant differences. Mid- and high-intensity ER at 935 MHz can reduce the fertilization rate in mice, and reduce the blastulation rate, thus reducing the possibility of embryo implantation.

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Cleavage; blastula; electromagnetic radiation; *in vitro* fertilization

Introduction

Mobile phones have become an essential daily communication tool, and the electromagnetic radiation (ER) they release fills our living space. Thus, the effects of this ER on health have always been a prominent subject of research. Studies have suggested that ER released by mobile phones could cause nervous system injury [1–5]. Between use, the phone is often placed at the waist, i.e., near the reproductive system, where germ cells are active in proliferation and metabolism as well as sensitive to changes in external environmental factors. Reproductive issues are directly related to congenital developments; therefore, the effects of ER released by mobile phones on reproduction have raised many concerns.

Some studies reported the effects of radiation on germ cells and progeny [6–14]. Interference with maturation, fertilization, embryo cleavage, and blastulation of an ovum can all affect pregnancy outcomes, and any factor that might affect development can have a negative effect on pregnancy. A previous study showed that simulated mobile phone ER might affect the Ca^{2+} concentration in ova, thus affecting their maturation and development [15]. The ER frequency used in that experiment was 935 MHz, simulating that widely used in mobile phones. In the present study, experimental groups were divided into low-intensity (mean intensity $150 \mu\text{W}/\text{cm}^2$), mid-intensity (mean intensity $570 \mu\text{W}/\text{cm}^2$), and high-intensity (mean intensity

1400 $\mu\text{W}/\text{cm}^2$) groups to further explore the effects of different ER intensities and exposure durations on fertilization and embryo development in mouse ova.

Materials and methods

Animals

As in the previous study [15], seven-week-old, sexually mature female KM mice (weighing 20 ± 2.5 g) and 10-week-old male KM mice (weighing 25 ± 3.2 g) were obtained from the Experimental Animal Center of Wuhan University (specific pathogen-free level), China. All animals were housed on a 12-h light and 12-h dark cycle in a temperature-regulated ($22 \pm 1^\circ\text{C}$) and humidity-controlled ($55 \pm 15\%$) animal room with water and food always available. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Wuhan University.

Electromagnetic radiation devices, duration, and intensity

Electromagnetic radiation devices consisted of four parts: a signal source (with frequency ranging from 935 to 960 MHz and magnetic field strength ranging from -15 db to $+15$ db), a rectifier (220 VAC/27 VDC; 300 W), a power amplifier, and a specific antenna with a length of 15 cm. The radiation container was home-made from plexi glass, with an inner diameter of 30 cm, an outer diameter of 46 cm, and a height of 15 cm. The cavity was symmetrically separated. The detection range of the monitor (EMR-300) was between 130 and 1500 $\mu\text{W}/\text{cm}^2$.

Radiation exposure and grouping

After adaptive feeding for a week, each female mouse received an intraperitoneal injection of 10 IU pregnant mare's serum gonadotropin (PMSG) (from a pharmaceutical plant in Inner Mongolia, China) at 1700 h to stimulate ovulation. After 48 h, each female mouse was injected intraperitoneally with 10 IU human chorionic gonadotropin (HCG) (from a pharmaceutical plant in Inner Mongolia, China). The mice were divided into seven groups by using a random table method: low-intensity (2 h/d and 4 h/d subgroups), mid-intensity ($570 \mu\text{W}/\text{cm}^2$: 2 h/d and 4 h/d subgroups), high-intensity ($1400 \mu\text{W}/\text{cm}^2$: 2 h/d and 4 h/d subgroups), and control groups. Every afternoon from the time of PMSG to HCG injection, ER at different intensities and exposure periods was continuously applied to each of the six study groups. The same experiment was carried out with sham-exposed animals, without energizing the device. The next morning, mice were sacrificed by cervical dislocation.

Preparation of in vitro fertilization (IVF) and embryo culture reagents

Dishes and broth were prepared the afternoon before the ovulation day. After sampling, the eggs were washed and placed in Quinn's fertilization solution plus 10% SPS in a fertilization dish; the growth medium used was Quinn's cleavage medium plus 10% SPS; then the eggs were covered with IVF-specific culture oil (Sage, Houston, TX, USA) for 16-h balancing in an

incubator (Forma, Waltham, MA, USA) at 37°C and 5.0% CO₂ (UJL Special Gas supply Co., Shanghai, China).

Collection of oocytes

Female mice were killed by cervical dislocation; the Fallopian tubes were cut and placed into egg-sampling dishes; a 1-mL BD syringe needle was then used to tear the pars ampullaris, and a sterile Pasteur pipette (Sigma, St. Louis, MO, USA) was used to collect the clumped eggs, which were then placed into individual dishes for washing; the washed eggs were then placed into fertilization dishes, with about 5 to 10 eggs in each droplet, for fertilization after incubation at 37°C and 5.0% CO₂ for 30 min.

Collection of sperm

Male rats were killed by cervical dislocation; the tail of the epididymis was then cut and placed in a 500-μL culture medium-containing EP tube for 1 h free of motion at 37°C and 5.0% CO₂. The supernatant was then sampled, placed into another EP tube, and centrifuged at 200 g for 10 min; the precipitate was then placed into the bottom of another 100–200-μL medium-containing EP tube for 1 h free of motion, and then held for further processing.

in vitro fertilization

A hemocytometer was used to count sperm density and observe vitality; a fine-drawn Pasteur pipette was then used to aspirate sperm, which were added to the fertilization droplet; the sperm density inside the fertilization droplet was then adjusted to $(1-3) \times 10^6/\text{mL}$, followed by incubation at 37°C and 5.0% CO₂ for 6 h.

Observation of normal fertilization

After a 6-h egg-sperm co-culture, a capillary pipette was used to repetitively disrupt granulosa cells; after washing, the fertilized eggs were transferred into growth dishes containing Quinn's cleavage + 10% SPS, and covered with IVF-specific culture oil; the balancing method was the same as before. Fertilization conditions were observed under an inverted microscope (200×, Nikon TE2000U, Japan); an egg with two pronuclei (2PN) was judged to be normally fertilized; 2PN rate is the ratio of those 2PN oocytes to all mature oocytes.

Observation of embryo cleavage

After observation on day 2 (d2), the embryos were transferred into pre-balanced blastula growth dishes containing Quinn's blastula medium + 10% SPS, and covered with IVF-specific culture oil; the balancing method was the same as before. After 72-h fertilization, the conditions of embryo cleavage were observed and scored. Embryo cleavage rate is the ratio of cleavage embryos to all 2PN embryos. When the embryo is compacting, that is the transformation of the embryo from a loose cluster of spherical cells into a tightly packed mass, we call it morula. The morula rate is the ratio of compacting embryos to all cleavage embryos. After 110-h fertilization, the conditions of blastulation were observed and scored. The blastula rate is the ratio of blastocysts to all cleavage embryos.

Table 1. Fertilization rate, morula rate, and blastulation rate after ER with different intensities and durations.

Culture stage	Control	2 h/d			4 h/d		
		Low	Middle	High	Low	Middle	High
2PN (%)	88.4 (99/112)	88.5 (108/122)	76.4 (81/106)	77.4 (89/115)	85.6 (107/125)	74.4 (90/121)	73.5 (97/132)
Morula (%)	72.7 (72/99)	72.2 (78/108)	63.0 (51/81) *	58.4 (52/89) *	74.8 (80/107)	53.3 (48/90)*, #	51.5 (50/97)*, #
Blastula (%)	65.3 (47/72)	70.5 (55/78)	54.9 (28/51) *	55.8 (29/52) *	62.5 (50/80)	52.1 (25/48)*, #	46.0 (23/50)*, #

Notes: Low-intensity: 150 $\mu\text{W}/\text{cm}^2$, mid-intensity: 570 $\mu\text{W}/\text{cm}^2$, high-intensity: 1400 $\mu\text{W}/\text{cm}^2$.

* Compared with the control group, $p < 0.05$.

Compared with the 2h/d subgroup within the same intensity group, $p < 0.05$.

2PN rate (2PN%) is the ratio of 2PN oocytes to all mature oocytes. The morula rate (morula%) is the ratio of compacting embryos to all cleavage embryos. Blastulation rate (blastula%) is the ratio of blastocysts to all cleavage embryos.

Statistical analysis

The data were processed using χ^2 analysis, and SPSS 16.0 software package was used for the analysis, with $p < 0.05$ considered as a statistically significant difference.

Results

As shown in Table 1, longer ER duration and increasing ER intensity resulted in a gradually decreasing fertilization rate, but no significant difference was found ($p > 0.05$). However, the morula rate and the blastulation rate in the mid- and high-intensity groups were decreased, and reached their lowest values in the high-intensity group, 4-h/d subgroup, exhibiting significant difference compared with the control group ($p < 0.05$). In mid- and high-intensity groups, the differences between 4-h/d and 2-h/d subgroups were significant ($p < 0.05$).

Discussion

Mechanisms of factors influencing fertilization and embryo cleavage

After passing through the zona pellucida, sperm enter oocyte with the aid of sperm-specific phospholipase C; the phospholipase C catalyzes the synthesis of 1,4,5 tris-trometamolphosphoinositide (IP3), which induces Ca^{2+} release from endoplasmic reticulum; the cytoplasmic Ca^{2+} concentration increases, resulting in periodic Ca^{2+} oscillations, and this Ca^{2+} oscillation terminates when the pronucleus is formed. During this process, the activation of oocyte begins at the sperm-egg plasma lemma fusion point, and spreads to the whole egg, thus inducing a transient Ca^{2+} fluctuation. Ca^{2+} thus triggering a series of intracellular morphologic and biologic changes, including oocyte detachment of MII block-age and the recovery of meiotic cell cycle, in turn inducing sister chromosome separation and pronucleus formation, mitotic cycle initiation, genome activation, maternal mRNA recruitment, and subsequent embryo development. Meanwhile, the sustained increase in intracellular Ca^{2+} triggers the exocytosis of cortical granules, thus preventing multi-sperm fertilization. Therefore, IP3-dependent Ca^{2+} plays an important role in the fertilization process. After fertilization, the zygote commences mitosis, thus splitting into small blastomeres, during which only DNA replication and cell division occur, while the total cells volume does not increase. The very early stage of cell division is synchronous; then the blastomeres merge and form different cell populations. These different cell populations continue splitting according to their unique characteristics until they generate specific cell lines. The stage at which the number of blastomeres reaches more than 16 cells is called the morula; at 32 cells,

the blastomeres differentiate and form the blastula. In the development process from morula to blastula, the embryonic metabolic activities change significantly, with more demand for nutrients. Studies [16–18] of the early mouse embryonic stage (2–8 cells) showed that the cell cycle, metabolism, and protein synthesis depend on the nutrition of oocyte, and the development after eight cells until the formation of the blastula stage depends on the activation of embryonic genome. If the early development of the gamete and embryo is interfered with by harmful factors such as ER, the activation of embryonic genome might be incomplete, or could not progress to the blastula stage; thus, embryo implantation may not be completed.

Many factors can influence the *in vitro* development of embryos, including egg and sperm quality, physicochemical factors, such as humidity and temperature, macro-environmental factors, such as air quality, or micro-environmental factors, such as medium droplet composition; macro-environment ultimately develops from micro-environmental factors, thus regulating embryonic development; these factors interact mutually, and change dynamically. Uncoordinated changes in one component will induce lethal effects in embryos. These factors include inorganic ions (Ca^{2+} , Mg^{2+} , etc.), bicarbonate, lactate, pyruvate, glucose, vitamins, antibiotics, chelating agents, hormones, macromolecules, essential and nonessential amino acids, and cytokines produced in the growth and development processes of embryos [16,17].

Roles of Ca^{2+} in fertilization and cleavage

Calcium plays an important role in the maturation process of egg nucleus; nuclear maturation is dependent on calmodulin and Ca^{2+} , which adjust phosphodiesterase activities within egg nuclei; thus enhancing the role of cAMP to achieve maturation. The release of Ca^{2+} is one of the earliest signs of meiosis. Since mammalian cell cycles are longer than those of marine organisms, this change in Ca^{2+} is only manifested as repeated or oscillatory stimulation to ensure the activation of oocytes as well as fertilization [18]. Eggs with ER-induced abnormal distribution of intra-cytoplasmic Ca^{2+} demonstrate corresponding changes in normal fertilization, but how the intracellular Ca^{2+} oscillation occurs requires further study. Meanwhile, because of the key role of cytoplasm activation in the egg cleavage process, the subsequent developmental qualities of embryos can also be affected.

In vivo, oocytes must undergo a series of physiological and biochemical changes to complete their maturation process, and simultaneously accumulate a large number of substances, such as mRNA, proteins, and a variety of other factors, to enable the development of fertilized zygote. Under the influence of ovulation stimulants, the nucleus can mature but the maturation of cytoplasm might be relatively inconsistent; thus, external physical or chemical factors could still have negative effects on IVF and embryo development. Oocyte nuclei might mature but immature cytoplasm might not complete the all process of fertilization; even if fertilized, eggs might demonstrate retarded developmental potential or embryonic death at early stages. Our previous studies have found that mobile phone ER might decrease the Ca^{2+} concentration in oocytes; this study revealed that the fertilization rate showed a decreasing trend with increasing ER intensity and time of exposure, which might be due to interference with the Ca^{2+} concentration inside the egg, in turn interfering with the fertilization process.

Effects of ER on embryonic quality

Oral et al. [19] reported that vitamins E and C could reduce the ER-induced oxidative stress of endometrial epithelial cells in peri-implantation stage, suggesting that vitamin-rich medium could also alleviate the radiation-induced stress on oocytes; however, the fertilization rate in this study was not significantly reduced by the effect of ER. Ethylene

diamine tetraacetic acid (EDTA) is a chelating agent for heavy metals and transition elements and acts as an antioxidant. EDTA in the culture medium could partially reduce the adverse effects caused by ER-induced Ca^{2+} abnormalities in the egg, but could not completely eliminate the effect, and the fertilization rate was decreased after irradiation [16]. Thus, nutrients in the culture medium could partially reduce the negative effects caused by radiation.

Compaction of morula is essential for blastula formation; it had been confirmed that Ca^{2+} not only participated in cellular physical activities but also played key roles in the compaction of morula. Therefore, ER-induced Ca^{2+} abnormalities in the egg could result in the incoordination of Ca^{2+} concentrations inside and outside the blastomeres, thus affecting the compaction of embryonic cells, reducing the morula rate, and further reducing the blastulation rate; under high-intensity, long-time irradiation ($1400 \mu\text{W}/\text{cm}^2$, 4 h/day), the morula rate and the blastulation rate in mice fell to their lowest level. Certain nutrients in the culture medium, such as essential and nonessential amino acids, significantly increased the blastulation rate. Alanine, glutamic acid, glycine, and glutamine promote the development of embryos at the cleavage stage; under low- and mid-intensity ER for 2 h/day or 4 h/day, the interactions of these nutrients might decrease ER toxicity; however, the morula rate and the blastulation rate showed no significant differences.

The results of this study showed that after ER, mouse eggs exhibited a declining trend in the IVF rate, and the blastulation rate was significantly reduced. This was consistent with the report by Borhani et al. [20–22], indicating that abnormalities occurring in the critical stages of egg development would have difficult recovering, even when protected from radiation, thus resulting in adverse effects in later development. The specific mechanisms and outcomes require further study.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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