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Detrimental effects of electromagnetic radiation emitted from cell phone on embryo morphokinetics and blastocyst viability in mice

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Research article

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Summary

Electromagnetic radiation (EMR) has deleterious effects on sperm motility and viability, as well as oocyte membrane and organelle structure. The aim was to assess the effects of cell phone radiation on preimplantation embryo morphokinetics and blastocyst viability in mice. For superovulation, 20 female mice were treated with intraperitoneal (IP) injections of 10 IU pregnant mare's serum gonadotropin (Folligon® PMSG), followed by 10 IU of human chorionic gonadotropin (hCG) after 48 h. The zygotes ($n = 150$) from the control group were incubated for 4 days. The experimental zygotes ($n = 150$) were exposed to a cell phone emitting EMR with a frequency range 900–1800 MHz for 30 min on day 1. Then, all embryos were cultured in the time-lapse system and annotated based on time points from the 2-cell stage (t2) to hatched blastocyst (tHDyz), as well as abnormal cleavage patterns. Blastocyst viability was assessed using Hoechst and propidium iodide staining. Significant increases ($P < 0.05$) were observed in the cleavage division time points of t2, t8, t10, and t12 of the experimental group compared with the controls. In terms of blastocyst formation parameters, a delay in embryo development was observed in the experimental group compared with the controls. Data analysis of the time intervals between the two groups showed a significant difference in the s3 time interval ($P < 0.05$). Also, the rates of fragmentation, reverse cleavage, vacuole formation, and embryo arrest were significantly higher in the experimental group ($P < 0.05$). Furthermore, the cell survival rate in the experimental group was lower than the control group ($P < 0.05$). Exposure to EMR has detrimental consequences for preimplantation embryo development in mice. These effects can manifest as defects in the cleavage stage and impaired blastocyst formation, leading to lower cell viability.

Introduction

It has been demonstrated that individuals are exposed to various low-level electric and magnetic fields, both in residential and occupational settings. These include the generation and transmission of electromagnetic waves by household appliances, workplace equipment, cell phones, and other devices. Cell phones, in particular, emit low levels of non-ionizing radiation, referred to as radiofrequency electromagnetic radiation (RF-EMR). If the RF-EMR radiation reaches a high level, it can have a thermal effect that raises body temperature and potentially gives rise to health issues (Gupta *et al*., 2022). Electromagnetic radiation (EMR) can affect human reproduction by affecting both male and female reproductive systems, as well as embryos and foetuses. Researchers have reported that exposure to EMR can potentially cause damage to various aspects of human reproduction, including spermatogenesis, oogenesis, and the post-fertilization stage (Altun *et al*., 2018; Yahyazadeh *et al*., 2018). Jaffar *et al.* (2019) suggested that EMR can have detrimental effects on sperm motility, viability, and cell membrane and organelle structure (Jaffar *et al*., 2019). In addition, EMR, including radiation emitted by cell phones, can cause different types of damage to chromosomes and the entire human genome (Jagetia, 2022). Santini *et al.* (2018) revealed that electromagnetic waves have the potential to harm cellular organelles, particularly mitochondria, by inducing oxidative stress (Santini *et al*., 2018). Damage to DNA structure is recognized as one of the primary issues related to male infertility. However, the precise threshold at which EMR can cause damage to DNA structure remains unclear.

Embryo implantation relies on two critical factors: a receptive uterus and the development of a competent blastocyst (Kim and Kim, 2017). Over the years, numerous efforts have been made to enhance the implantation success rate in assisted reproductive technology (ART). One such approach involves the utilization of a more reliable morphokinetic measurement method to select a competent embryo, as opposed to traditional methods based solely on morphological criteria (Szekeres-Bartho, 2016). Previous studies have also indicated the harmful effects of cell phone waves on gametogenesis and embryo quality in laboratory models (Safian *et al*., 2016;

Mahaldashtian *et al*., 2022). The development of techniques, such as time-lapse imaging coupled with artificial intelligence, has emerged as a valuable tool for embryo culture and selection (Lundin and Park, 2020). Furthermore, scientists have directed their attention to other areas of interest, including the impacts of radiation emitted by cell phones on the biological integrity of embryos (Mahaldashtian *et al*., 2022).

Multiple studies have provided evidence that EMR, including radiation from cell phones, can cause different forms of damage to embryo development and the entire genome (Jagetia, 2022). In recent years, the effects of EMR have been assessed on embryos and implantation in various organisms (Üstündağ *et al*., 2020; Yenilmez, 2022; Augustianath *et al*., 2023). The objective of the current study was to investigate the effects of cell phone radiation on the embryo morphokinetics, and blastocyst viability in mice. According to our knowledge, this study for the first time assessed the morphokinetics of embryo development following exposure to cell phone radiation to *in vivo*-formed zygotes in mice.

Materials and methods

In total, 30 NMRI mice, consisting of 20 females and 10 males, aged 6–8 weeks, were obtained from the animal house of our institution (IIR.SSU.MEDICINE.REC.1400.314). All procedures contributing to this study were done under the supervision of the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals. Mice were housed under temperature-controlled conditions with a 12 h:12 h, light:dark cycle and free access to food and water.

Zygote preparation

For induction of superovulation, 6-week-old female mice were treated with intraperitoneal injections of 10 IU Folligon® PMSG (Gonaser, Hipra, Spain), followed by 10 IU of human chorionic gonadotropin (hCG) after 48 h. Two females were then allowed to mate overnight with a male mouse. The success of mating was confirmed by the presence of a vaginal plug approximately 20 h later. Mice were then euthanized through cervical dislocation, and the ovaries were surgically removed to extract the ovarian bursa, which was placed in warm Hams' F10 medium. Using a stereomicroscope (Olympus, SZX10, Japan), zygote dissection was performed from the swollen ampulla. The collected zygotes were transferred into a one-step medium (SAGE 1-Step, Origio Co., Denmark), followed by immediate incubation under standard conditions in an incubator (37°C, 5% CO_2).

Two groups were included: control ($n = 150$ zygotes) and experimental ($n = 150$ zygotes) groups. The embryos in the experimental group were exposed to radiofrequency (RF) radiation for 30 min on day 1. The embryos were cultured for a maximum of 4 days in a one-step medium, covered with liquid paraffin. The developmental progress of the embryos, including morphokinetic aspects, was monitored daily using time-lapse microscopy (Primo vision, Vitrolife Kft., Sweden). The undesired events, such as abnormalities, were recorded during the observation period.

The zygotes in the experimental group were exposed to EMR emitted by a commercial cell phone, with the following characteristics: a specific absorption rate (SAR) of 0.683–0.725 W/kg and a frequency range 900–1800 MHz. To expose the zygotes, the cell phone was positioned horizontally inside the incubator, parallel to the culture medium. During the exposure period, the cell phone remained continuously in talk mode and was kept at a distance of more than 10 cm from the zygotes. The zygotes were stabilized in the incubator for 1 h before being exposed to electromagnetic waves from the phone for 30 min on day 1. Subsequently, the zygotes were transferred to the time-lapse dish and placed in the incubator. The same process was carried out for the control group; however, the exposure step was omitted.

Assessment of cell viability

Cell viability was assessed using Hoechst and propidium iodide (PI) staining. For the identification of viable cells, the blastocysts were stained with PI and the H33258 method. The stained blastocysts were washed twice with PBS buffer and then incubated for 30 min in a staining solution containing PI (300 µg/ml) and Hoechst (5 µg/ml) that had been pre-equilibrated (Chatroudi *et al*., 2019). For the removal of residual dye, the embryos were washed three times with warm PBS. Subsequently, the stained embryos were fixed in 2.5% glutaraldehyde for 5 min at room temperature, followed by another round of washing. For mounting, the fixed embryos were placed in a drop of glycerol between two lines of paraffin wax. Cell viability was observed under a fluorescence microscope (Olympus, IX71, Japan, DAPI and Texas red filters), where necrotic cells appeared red, whilst live cells exhibited a dark blue colour.

Embryo quality

The cleaved embryos were graded according to a reported grading system (Anbari *et al*., 2021): Grade A: identical blastomeres without fragmentation; Grade B: almost equal blastomeres, up to 10% cytoplasmic fragments; Grade C: unequal blastomeres with as many as 50% fragments and massive granules; and Grade D: unequal blastomeres with huge fragmentation and black granules. Notably, in this approach, Grades A and B are considered high-quality embryos, whereas Grades C and D are considered low-quality embryos.

In vitro culture and time-lapse microscopy

On the fourth day of culture, all embryos that were cultured in the time-lapse system were analyzed retrospectively by an embryologist using the embryo viewer software (Vitrolife). The recorded videos of the embryos were manually examined to assess various variables related to early cleavage kinetics. Time of pronuclei disappearance (tPNF), time from the formation of the second to twelfth discrete cells (t2–t12), times of early compaction (tSC), full compaction (tMF), early blastocyst (tSB), full blastocyst (tByz), blastocyst hatching (tHNyz), hatched blastocyst formation (tHDyz), duration of the second cell cycle (cc2: t4–t2), Synchronization of cell division (s2: t4–t3), duration of the third cell cycle (cc3: t8–t4), synchronization of cleavage pattern (s3: t8–t5). Furthermore, the final morphological stage of the embryos was evaluated on days 4–5.

Data analysis

Sata analysis was conducted using the Statistical Package for the Social Sciences software version 20 (SPSS, Inc., Chicago, IL, USA). The Shapiro–Wilk test was used to assess normality. For statistical analysis, both Student's *t*-test and the Mann–Whitney *U*-test were utilized. A significance level of $P < 0.05$ was considered significant. Plots were generated using GraphPad Prism 8.0.1 (GraphPad Software, Inc.).

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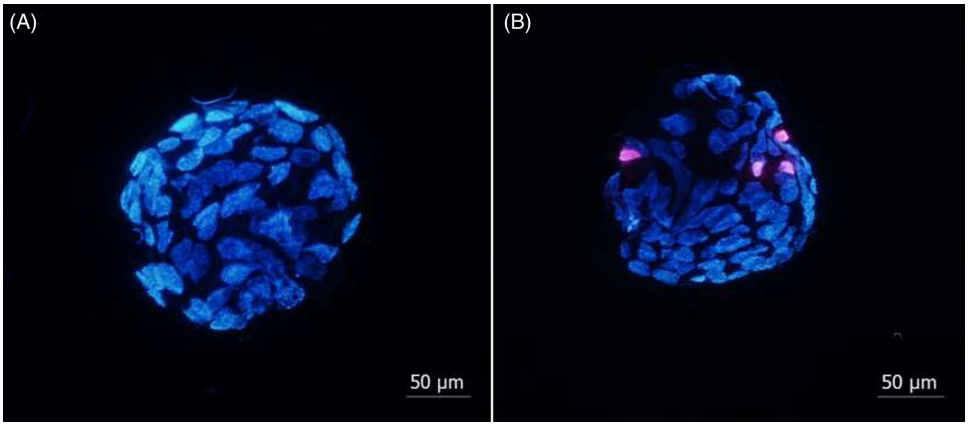

Figure 1. Cell viability evaluation using Hoechst and propidium iodide (PI) staining. (A) Control group, all cells are viable, as indicated by the dark blue colour. (B) Experimental group, necrotic cells are shown in red, whilst live cells are represented by the dark blue colour.

Results

Blastocyst cell viability using Hoechst and PI staining is presented in Figure 1. Our results showed significant differences in division times between the experimental and the control groups at the specific time points of t2, t8, t10, and t12 (Figure 2). In addition, data analysis revealed a significant difference between the experimental and control groups in terms of cavitation (blastocoel formation), tSB (time of early blastocyst formation), tByz (time of full blastocyst formation), tHNyz (time of blastocyst hatching), and tHDyz (time of hatched blastocyst formation). In these stages, significant delays were observed in the experimental group ($P < 0.05$; Figure 3).

Data analysis of the time intervals between the control and experimental groups showed a significant difference ($P < 0.05$) in only one interval, s3, as depicted in Figure 4. A comparison of cleavage abnormalities between the control and experimental groups showed that the rates of fragmentation, reverse cleavage, vacuole formation, and embryo arrest were significantly higher in the experimental group compared with the control ($P < 0.05$). However, no significant differences were observed between the two groups in terms of direct cleavage (Figure 5).

However, at the 8-cell, full blastocyst, hatching, and hatched stages, the numbers of embryos were significantly lower in the experimental group compared with the control ($P < 0.05$; Figure 6). Table 1 presents the blastocyst viability analysis. The data indicated that the cell survival rate in the experimental was lower (88.37%) compared with the control group (94.34%).

Discussion

In the present study, our focus was to investigate the effects of cell phone radiation on morphokinetics, cell viability, and embryo quality at the preimplantation stage. Analysis of time-lapse imaging showed a delay in cleavage at time points t2, t8, t10, and t12 in the experimental group compared with the control. These results suggested that exposure to EMR can lead to embryonic defects in the early stages after fertilization. Furthermore, except for the delay observed at time t2, it appears that the SAR of EMR, ranging from 0.683 to 0.725 W/kg, and the frequency of 900–1800 MHz, are time-dependent characteristics under the conditions of the current study. This finding confirms the claims made in a previous review regarding the biological responses to mobile phone exposure frequencies (Maalouf *et al.*, 2023). In their study, two types of damage, namely thermal and non-thermal, were investigated during exposure to cell phones. The SAR of radiofrequency radiation (RFR) was introduced as an indicator of thermal damage, whilst the time-dependent factor was considered as a parameter in assessing non-thermal effects. Chen *et al.* (2021) conducted a study on the effects of electromagnetic waves on oocyte maturation and embryonic development in pigs. Their results indicated that there was no significant difference in oocyte maturation rate. However, the cleavage rate and the number of blastocysts derived from oocytes treated with low-intensity EMR showed a significant decrease compared with the control group (Chen *et al.*, 2021). Our results confirmed the recent findings reported by Chen *et al.* (2021). It is worth noting that the present study focused on mouse embryos, whilst they examined pig embryos. Despite the differences in the experimental subjects, both studies yielded consistent results. In both cases, the evidence pointed to the detrimental effect of electromagnetic waves on the cleavage rate and embryo development.

There is limited available information regarding the effect of electromagnetic waves on blastocyst formation. In a related study, a negative effect of intermediate frequency electromagnetic fields (IF-EMF) was noted on blastocyst formation. However, their claims remained at a general level and did not provide sufficient details (Chen *et al.*, 2021). In contrast, our analysis confirmed that during the compaction stage, specifically early and full compaction, electromagnetic waves significantly delayed the process of blastomere formation. Furthermore, our findings indicated that this delay persisted throughout all stages, from cavitation to blastocyst hatching, in the experimental group. These results demonstrated the negative effect of EMR on cleavage and cell division, consequently impacting blastomere formation from cavitation to hatching blastocyst. This developmental complication may ultimately result in embryonic abnormalities.

In addition, the rates of fragmentation, reverse cleavage, vacuole formation, and embryo arrest in our work were significantly higher in the experimental group. It is suggested that EMR exposure may contribute to the production of ROS in various tissues of the human body, including the reproductive system

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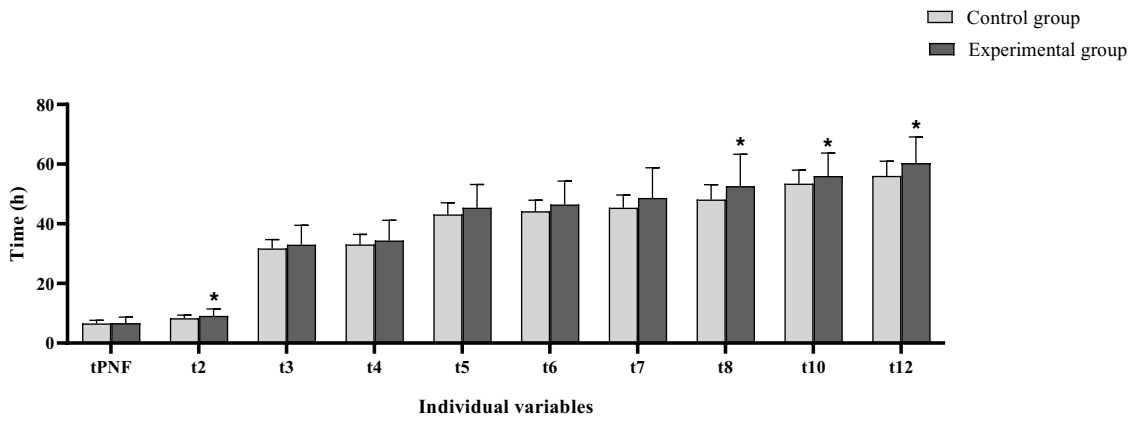

Figure 2. Individual morphokinetic variables of cleavage divisions. Time-lapse analysis highlights significant differences in cleavage divisions between the two groups at specific time points. Notably, at time points t2, t8, t10, and t12, a significant delay was observed in the experimental group compared with the control ($P < 0.05$).

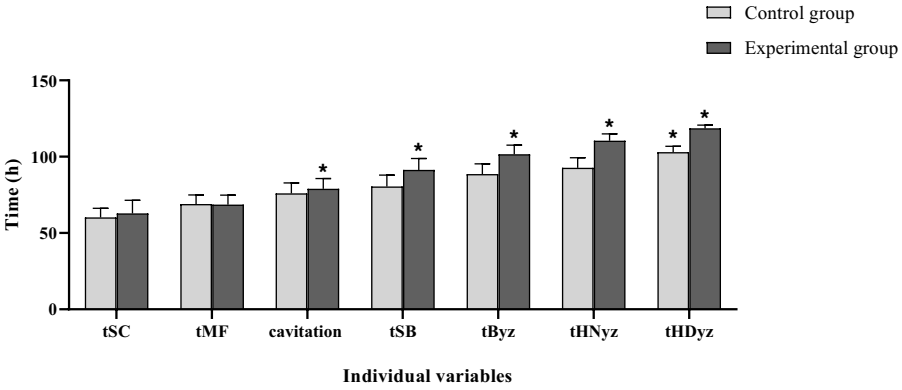

Figure 3. Comparison of individual morphokinetic variables in blastocyst formation between the experimental and control groups. Significant changes were observed ($P < 0.05$). The variables are as follows: tSC: Early Compaction, tMF: Full Compaction, cavitation: Formation of Blastocoel, tSB: Early Blastocyst, tByz: Full Blastocyst, tHNyz: Hatching Blastocyst, tHDyz: Hatched Blastocyst.

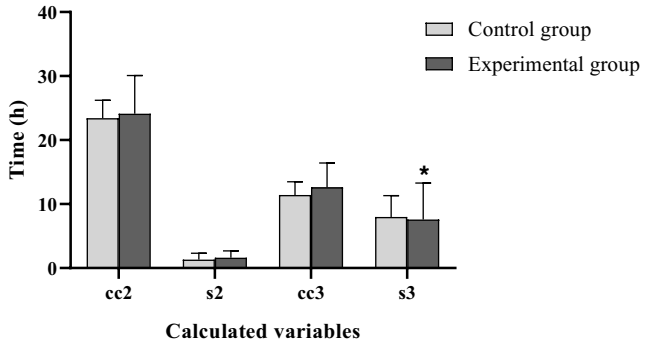

Figure 4. Comparison of time intervals between the control and experimental groups. A significant difference ($P < 0.05$) was observed specifically in the s3 time interval.

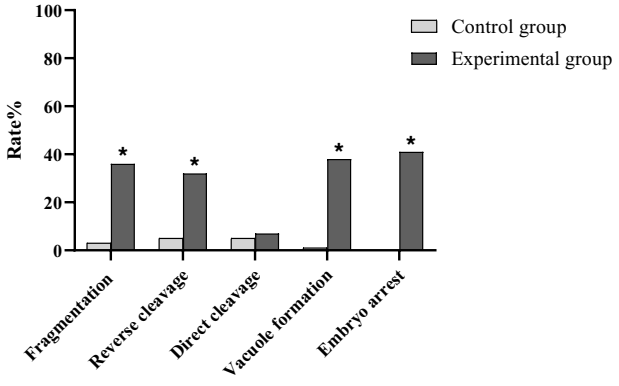

Figure 5. The rates of fragmentation, reverse cleavage, vacuole formation, and embryo arrest in the experimental group were significantly more than those in the control group ($P < 0.05$). Data are presented as number (%) of observed cases. No significant difference was observed between the two groups in terms of direct cleavage.

(Altun *et al.*, 2018). Furthermore, the production of ROS induced by EMR can lead to damage to mitochondrial function. This impairment results in inadequate ATP production and errors in embryo development. Additionally, ROS can trigger apoptotic-related events, such as DNA fragmentation and altered gene expression (Soto-Heras and Paramio, 2020). Our recent study demonstrated that EMR exposure during zygote development, which led to ROS induction, significantly decreased blastocyst development in mice. Moreover, in the experimental group, there was an upregulation of mRNA levels of heat shock protein (*Hsp70*), and a downregulation of the *Sod* gene, which is involved in antioxidant defence (Koohestanidehaghi *et al.*, 2023). Therefore, it appears that, in addition to impairing cleavage and blastocyst formation, EMR may also contribute to the failure of successful implantation through sub-structural damage to early-stage embryos.

In addition, we observed a reduction in blastocyst cell viability in the experimental group. EMR exposure caused changes in the membrane integrity, leading to an induction of membrane

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Table 1. Comparison of the blastocyst cells viability in two groups

Viability analysis	Control	Experimental	*P*-value
Live cells	52.86 ± 6.14	49.3 ± 5.35	0.02*
Dead cells	3.13 ± 1.59	6.43 ± 1.16	0.00*
Viability rate	94.39 ± 2.8	88.37 ± 2.26	0.00*

Values are presented as the mean ± standard deviation (SD). The viability rate was calculated by dividing the number of living cells by the total number of cells and multiplying the result by 100. *Showed significant differentation between 2 groups.

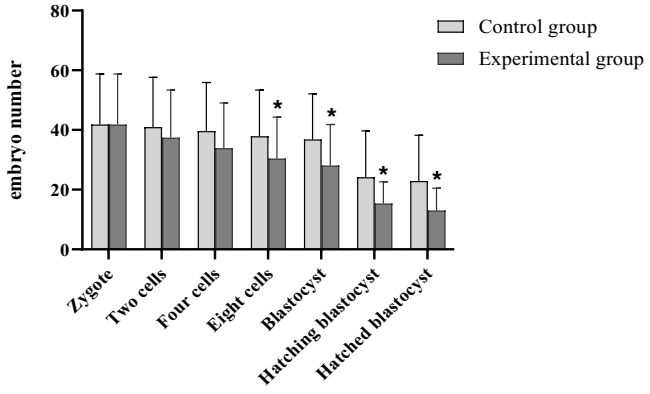

Figure 6. Fewer embryos were observed in the experimental group compared with the controls at the 8-cell, blastocyst, hatching, and hatched stages ($P < 0.05$).

permeability. The increased permeability allowed the penetration of PI into the cells. Consequently, this event resulted in decreased membrane stability and reduced viability in the experimental group (Kim and Kim, 2017). This finding indicates that the effects of EMR not only disrupt different stages of embryo development but may also lead to complete cell death in subsequent embryo development. These findings confirmed the results of our previous study, in which we investigated the survival of mouse embryos exposed to cell phone radiation during the preimplantation stage (Safian *et al.*, 2016).

In conclusion, the results suggested that exposure to EMR during the preimplantation embryo stage had detrimental consequences on embryo development and morphokinetic parameters. These adverse effects encompass defects in cleavage, impaired blastocyst formation, delayed cleavage events, and reduced cell viability. Further comprehensive studies should be conducted in this field to obtain indisputable results.

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