Zygote

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Challenges on the effect of cell phone radiation on mammalian embryos and fetuses: a review of the literature

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Review Article

Cite this article: Mahaldashtian M *et al*. (2022) Challenges on the effect of cell phone radiation on mammalian embryos and fetuses: a review of the literature. *Zygote*. **30**: 176–182. doi: 10.1017/S0967199421000691

Received: 23 April 2021
Revised: 2 August 2021
Accepted: 2 August 2021
First published online: 29 September 2021

Keywords:
Cell phone; Embryo development; Fetus; Mammal; Radiofrequency-electromagnetic radiation (RF-EMR)

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Summary

Cell phones operate with a wide range of frequency bands and emit radiofrequency-electromagnetic radiation (RF-EMR). Concern on the possible health hazards of RF-EMR has been growing in many countries because these RF-EMR pulses may be absorbed into the body cells, directly affecting them. There are some *in vitro* and *in vivo* animal studies related to the consequences of RF-EMR exposure from cell phones on embryo development and offspring. In addition, some studies have revealed that RF-EMR from cellular phone may lead to decrease in the rates of fertilization and embryo development, as well as the risk of the developmental anomalies, other studies have reported that it does not interfere with *in vitro* fertilization or intracytoplasmic sperm injection success rates, or the chromosomal aberration rate. Of course, it is unethical to study the effect of waves generated from cell phones on the forming human embryos. Conversely, other mammals have many similarities to humans in terms of anatomy, physiology and genetics. Therefore, in this review we focused on the existing literature evaluating the potential effects of RF-EMR on mammalian embryonic and fetal development.

Introduction

Radiofrequency-electromagnetic radiation (RF-EMR) is a general term that describes periodic changes between positive and negative, and the speed or the number of changes per second, called the frequency, with Hertz as units (Beraldi *et al*., 2003). RF-EMR generated from mobile phones has frequency radiation between 900–1800 MHz (Safari *et al*., 2020). Specific absorption rate (SAR) is the amount of RF energy absorbed by a person's body during a phone call and usually is recorded in units of watts per kilogram (W/kg) or in milliwatts per gram (mW/g) (Gautam *et al*., 2019). Most phones have SAR ranging from 0.1 to 1.2 W/kg (Safian *et al*., 2016). In assisted reproductive technology (ART), embryos are generated and cultured under *in vitro* conditions. Therefore, environmental disruptions, such as RF-EMR exposure, may affect their survival and developmental capacities, therefore concerns about risk of malformations after being subjected to cell phone radiation have been increasing (Beraldi *et al*., 2003).

There have been some *in vitro* and *in vivo* animal studies that assessed the effects on embryogenesis and newborns of RF-EMR propagated from cell phones. Some experiments revealed that RF-EMR from cell phones did not induce significant changes in the rates of fertilization and embryogenesis (Suzuki *et al*., 2017), in addition to histological or ultrastructural changes in embryos (Klug *et al*., 1997). In contrast, other studies have indicated that this radiation may cause decrease in embryo quality and blastulation rates and an increase in the number of embryo deaths (Fatehi *et al*., 2018). From these experiments, it may be concluded that RF-EMR can be considered an environmental stress factor. Exposure of cells to stressful states may be associated with increased synthesis of stress proteins, including heat-shock proteins (Hsp), and increased levels of reactive oxygen species (ROS) (Weisbrot *et al*., 2003; Safian *et al*., 2016). It has been confirmed that increased levels of ROS have detrimental effects on the embryo and fetus (Lampiao, 2012; Shafiei *et al*., 2020). RF-EMR could induce numerous anomalies in offspring (El-Sayed *et al*., 2011; Celikozlu *et al*., 2012; Haghani *et al*., 2013; Ye *et al*., 2016), even though these effects may be temporary (Fragopoulou *et al*., 2010).

As other mammals have many similarities to humans in terms of anatomy, physiology and genetics, the aim of this article was to summarize the existing literature that assessed the influences of RF-EMR exposure from cell phone on fertilization and subsequent embryonic and fetus development in other mammals *in vitro* and *in vivo*.

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Table 1. Effects of RF-EMR emitted from cell phone on embryo development in mammals

Model	Intensity	Frequency	Exposure time / day	SAR	Cell -phone distance	Results
Wistar rats	600 W/m	900 MHz	Up to 36 h	0.2, 1 and 5.0 W/kg	–	No histological or ultrastructural changes observed in embryos exposed to electric or magnetic fields (Klug *et al.*, 1997)
BALB/c mice	–	900–1800 MHz	30 min/day	0.683 to 0.725 W/kg	>10 cm	No major adverse effects on embryo development. Increased rates of dead cells in blastocysts and changes in membrane permeability and apoptosis (Safian *et al.*, 2016)
B6D2F1 mice		1.95 GHz	60 min/day	2 mW/g	–	Fertilization and embryogenesis rates were insignificant (Suzuki *et al.*, 2017)
KM mice	150,570 and 1400 μW/cm^2	935–960 MHz	2 or 4 h/day	–	–	Mid and high intensity reduced the fertilization and blastulation rates (Chen *et al.*, 2017)
NMRI mice	–	900 MHz	1, 5 and 10 h/day	–	10 cm	Decreased quantity of 2-cells embryos, grade A embryos and number of newborn mice. Also, increased fragmentation, grades C and D embryos and pregnancy duration resulting in fertility failure (Fatehi *et al.*, 2018)

Materials and methods

We searched MEDLINE–PubMed (http://www.ncbi.nlm.nih.gov/PubMed), Google Scholar (https://scholar.google.com/), Scopus (https://www.scopus.com) and ISI Web of Science (http://apps.lib.wosg.ir/WOS) databases, from the earliest available online indexing year until April 2021 using the following Medical Subject Headings (MeSH) and non-MeSH keywords: 'cell phone', 'embryo development', 'mammals', and 'fetal'. The searches were limited to original articles published in English and examined the *in vitro* and *in vitro* effects of RF-EMR emitted from cell phones on mammalian embryonic and fetal development. Other animal studies were excluded from this review.

Results

Effects of RF-EMR on embryo development

There have been few studies with different protocols that assessed the consequences of cell phone radiation on embryo development (Table 1). Klug *et al.* (1997) exposed 11.5-day-old rat embryos (two or three somites) to RF-EMR. After 48 h, the rates of embryo growth and differentiation were evaluated using a scoring system. Both histological examination and protein content of the embryos were determined. They suggested that exposure to electric or magnetic fields did not significantly influence normal growth and differentiation of rat embryos *in vitro* (Klug *et al.*, 1997). Also, Safian and co-workers (2016) evaluated the effects of cell phone radiation on *in vitro* development of mouse embryos. In their study, two-cell embryos were exposed to RF-EMR for 30 min/day for 4 days consecutively. The quality of embryos was documented daily and fluorescence staining was used for identification of viable blastocysts. Based on their results, both embryo survival and blastocyst formation rates were similar in both groups. However, death of embryos at the 2-cell stage and loss of cell viability in blastocysts were significantly higher in the experimental group (Safian *et al.*, 2016). In a study administered by Suzuki and associates, both spermatozoa and oocytes were separately exposed to RF-EMR for 60 min. Then, *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) were performed using combinations of exposed and non-exposed oocytes plus spermatozoa. Interestingly, their results showed that waves propagated from cell phones had no significant detrimental effects on the rates of chromosomal abnormality, as well as IVF and ICSI fertilization rates, even though the amount of exposure in their experiment was 100 times greater than regular exposure to human spermatozoa and oocytes (Suzuki *et al.*, 2017).

In a recent study, ovulating mice were irradiated at three RF-EMR intensities low intensity (mean intensity 150 μW/cm^2), mid intensity (mean intensity 570 μW/cm^2), and high intensity (mean intensity 1400 μW/cm^2) for 4 or 2 h/day for 3 consecutive days. After that, the ova were collected for the IVF protocol. Their results indicated a decline in the IVF rate in the treatment groups compared with the control (Chen *et al.*, 2017). Also, Fatehi and colleagues (2018) separated male mice into five groups as control (I) and experimental groups (II–IV). Groups II, III and IV were exposed to cell phones on 'Standby mode' for 1, 5 and 10 h/day, respectively. Group V, however, was exposed to a cell phone on 'Active mode' 1 h/day. At 30 days after treatment, the mice were sacrificed and IVF was performed subsequently. Based on their studies, RF-EMR for 1 h on Active mode had more damaging effects compared with the 1 h Standby mode. Also, increased exposure time from 1 h to 5 h and 10 h, resulted in a higher decrease in the numbers of two-cell embryos and blastocyst rates (Fatehi *et al.*, 2018).

Effects of RF-EMR on offspring

Some studies focused on offspring following daily exposure of pregnant animals near to the RF-EMR emitted by a mobile phone (Table 2). Ogawa and her teammates subjected 7–17-day-old gestational rats to RF-EMR. In their study, the prenatal rats were allocated into four groups, with five pregnant rats in each group. Group 1 (control): animals were kept in conventional cages. Rats in Groups 2–4 were positioned in the exposure chamber for 90 min/day in the morning: Group 2 was considered as sham. Groups 3 and 4 were subjected to RF-EMR at low (average SAR: 0.67 W/kg) and high (average SAR: 2.0 W/kg) levels, respectively. Based on their results, exposure of the animal's head to weak mobile phone-like signals during gestation induced no significant

https://doi.org/10.1017/S0967199421000691 Published online by Cambridge University Press

Table 2. Effects of RF-EMR emitted from cell phone on offspring in mammals

Model	Intensity	Frequency	Exposure time/day	SAR	Cell phone distance	Results
Rats		1.95 GHz	90 min/day	0.67 and 2 W/kg	Distance between the antenna and each rat nose was set at 30 mm; and the distance between the ceiling and each rat's back was 5 mm	There were no harmful effects of RF-EMR exposure on reproductive parameters including the number of live, dead or resorbed embryos, placental weights, sex ratios, weights or external, visceral or skeletal abnormalities of live fetuses (Ogawa *et al.*, 2009)
BALB/c mice	–	900 MHz	6 and 30 min/day	0.6–0.94 W/kg	Middle of the cage	In mild (6 min daily) RF-EMR throughout the gestation period, bone formation of fetuses was reversibly affected, therefore normal osteogenic pattern shown by 35-day-old mice. Histological explanations did not show any significant differences (Fragopoulou *et al.*, 2010)
Rats	–	1800 MHz	30 min/day	2.02 W/kg	Less than 10 cm	Decrease in the mother's and fetal body weights and increase in the abortion rate and skeletal system abnormalities observed in treated groups. Also, expression levels of *Msx1* and *Cx43* were affected by the treatment (El-Sayed *et al.*, 2011)
Rats	–	–	3 times a days; each time 10 (low), 30 (medium), 60 (high)	0.9 W/kg	–	Changes in fetal rat brain, not only OS system, but also neurotransmitters. In medium- and high-dose groups, the contents of SOD and GSH-Px decreased and the content of MDA increased. In fetal rat brains of the low-dose group, increase in the content of NE and DA was observed; while, it was decreased in the high-dose group (Jing *et al.*, 2012)
Albino Wistar Rats	–	900MH	24 h and 30 min/day	–	–	Increased blood glucose and serum protein level as well as ischaemic neuron numbers, but pyramidal neuron numbers decreased. TRF-EMR affected some biochemical parameters, especially the cortex region of the brain (Celikozlu *et al.*, 2012)
Rats		900 MHz	6 h/day	0.5 and 0.9 W/kg		Series of disruptions in electrophysiological properties of Purkinje cells; but, behavioural abnormalities not observed (Haghani *et al.*, 2013)
Rats		900–1800 MHz	60 min/day	0.1 W/kg	1 cm	Induced OS in the kidney and testes by enhanced level of lipid peroxidation and reduced level of antioxidant (Özorak *et al.*, 2013)
Sprague–Dawley rats	–	900MH	24 h/day	2 W		Mild histopathological effects on the kidneys of prenatal rats and mild biochemical changes in the blood of offspring (Bedir *et al.*, 2015)
Wistar albino rats	11 V/m	900–1800 MHz	60 min/day	0.1 W/kg	10 cm	Reduced levels of prolactin, oestrogen and progesterone in the plasma of maternal rats and their offspring, while the plasma total oxidant status and body temperatures increased (Yüksel *et al.*, 2016)
Rats	–	1800 MHz	1 and 2 h/day	0.048 W/kg		Detrimental effects on pregnancy results and delay in normal, functional and physiological postnatal development in cubs (Alchalabi *et al.*, 2016)
BALB/c mice	1 mW/m^2	900–1800 MHz	2 h/day	–	2 m	OS in tissues of both the mothers and their offspring (Bahreyni Toossi *et al.*, 2018)
Wistar Rats	–	900 MHz	6 h/day	–	–	No pregnancy period changes noted in control and cell phone-exposed rats (Haghani *et al.*, 2020)

https://doi.org/10.1017/S0967199421000691 Published online by Cambridge University Press

developmental changes in the offspring (Ogawa *et al.*, 2009). Furthermore, Fragopoulou and co-operators exposed 5-day pre-pregnancy female mice to RF-EMR. The 5-day period before pregnancy exposure was proposed to permit stimulation of the intrinsic defence system. After positive mating (vaginal smear), pregnant mice were exposed again until term (12–14 days).

All the mice in both control and experiment groups were weighed regularly, and observed daily throughout pregnancy for overall appearance and locomotor activity by cage-side observation. At 5 h after birth, most pups were fixed for histological assessment and others remained for structural assessment. Their data indicated that mild exposure to RF-EMR from cell phones adversely influenced mice fetal development at the ossification level (Fragopoulou *et al.*, 2010). Also, El-Sayed and teammates exposed pregnant rats to RF-EMR from the 1st to 20th and 7th to 16th days of pregnancy. At the 20th day, the fetuses were detached from the uterus and evaluated. Liver of pregnant rats and their cubs were used for extraction of total RNA for quantification of *Msx1* and *Cx43* genes using real-time PCR. They noticed that subjecting to the rats to cell phones throughout the gestational period changed gene expression as well as some morphological and physiological factors in prenatal rats and their fetuses (El-Sayed *et al.*, 2011).

In a study conducted by Jing and colleagues, pregnant rats were exposed to different intensities of RF-EMR. Experimental groups were irradiated three times a day from the 1st day of gestation for consecutively 20 days. On the 21st day, the fetuses were removed and the levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), noradrenaline (NE), dopamine (DA), and 5-hydroxyindole acetic acid (5-HT) in the brain were examined. The distance between the antenna and pregnant rat's ear completely mimicked human methods of phone calling. Also, throughout the call, the antenna of the phone was kept near to the ear. They agreed that RF-EMR from cellular phones during the prenatal period had adverse effects on rat fetal brains (Jing *et al.*, 2012). In the same year, Celikozlu and colleagues evaluated the influence of magnetic fields from cell phones on some blood parameters and neurons in the brain of rats. The cell phones were located on the wall of the cage and were in standby mode for an entire day and in talk mode for 30 min per day. Rats were exposed to RF-EMR during prenatal and postnatal periods until they were 80 days old. Their findings proved that exposure to cell phones influenced some biochemical factors, particularly in the cortex region of the brain (Celikozlu *et al.*, 2012).

In 2013, Haghani and co-workers exposed female rats to RF-EMR throughout the pregnancy period. Then, the newborns were evaluated for behavioural and electrophysiological status. Their outcomes demonstrated that prenatal RF-EMR exposure led to altered electrophysiological properties in Purkinje neurons. These modifications, however, were not serious enough to modify cerebellum-dependent functional properties (Haghani *et al.*, 2013). In another research study, rat cubs were exposed to RF-EMR from the beginning of pregnancy until 6 weeks after births. During the 4th to 6th weeks of the research, kidney and testis of offspring were removed and the levels of lipid peroxidation (LPO), copper, zinc, GSH, GSH-Px and total antioxidant status (TAS) were assessed. The data showed that waves from mobile phones led to precocious puberty and oxidative damage to the kidneys and testes of the rats (Özorak *et al.*, 2013).

In addition, Bedir and colleagues irradiated rats with cell phone on 'talk mode' during first 20 days of the gestational period for 24 h per day. They evaluated histopathological variations and apoptosis in the kidneys, together with the levels of urea, creatinine and electrolytes in serum. The histological samples were assessed using immunohistochemistry. Their outcome indicated that RF-EMR emitted from cell phones had damaging effects on the renal development of pregnant rats (Bedir *et al.*, 2015).

Yüksel and team workers subjected pregnant rats (60 min/day for 5 days a week) for four generations. At the beginning of the pregnancy, female adults were irradiated for an additional 3 weeks. After specifying the sex of the newborns, the female progenies were exposed to additional radiation up to 6 weeks of age. They showed that mobile phones induced changes in progesterone, oestrogen and prolactin concentrations, oxidative stress (OS) levels, and endocrine functions in growing and maternal rats. Also, EMR could be a source of uterine injury in growing rats (Yüksel *et al.*, 2016).

Alchalabi and teammates also exposed pregnant rats to RF-EMR from cell phones in the 1st to 3rd weeks of pregnancy. They saw uterine congestion, haemorrhage, and dead and reabsorbed fetuses during the 2nd and 3rd weeks of pregnancy and a meaningful decrease in the implantation sites and embryos during the 1st and 2nd weeks (Alchalabi *et al.*, 2016). The effects of RF-EMR exposure to mobile phones during the gestational period on some biomarkers of OS were assessed in tissues, such as heart, liver, kidney, and the cerebellum of offspring mice. Based on the results, increased levels of MDA and decreased levels of total thiol groups (TTG), SOD, and catalase (CAT) were observed in all tissues from both mothers and offspring (Bahreyni Toossi *et al.*, 2018).

More recently, Haghani and colleagues exposed prenatal rats to cell phone radiation during pregnancy for 6 h/day. Their results indicated that exposure to cell phone radiation did not induce any significant alternations in body temperature, litter size, length of gravidity, body weight of newborns and mortality rate between control and experimental groups. However, it damaged the regular current flow of the cerebellar Purkinje neurons (Haghani *et al.*, 2020).

Discussion and limitations of studies

Cell phones are an indispensable method of communication in our daily lives. However, there are worries about the healthcare effects from RF-EMR emitted from cell phones. Epidemiological studies have suggested that RF-EMR from cellular phones may increase the risk of brain tumours (Sato *et al.*, 2011), diabetes (Sakurai *et al.*, 2004), sleep and personality disorders (Abdel-Rassoul *et al.*, 2007). In addition, some studies have demonstrated an association between RF-EMR exposure and human reproductive diseases. In reproduction technology, there has been a focus on the effects of RF-EMR exposure on sperm parameters (Gutschi *et al.*, 2011; Okechukwu, 2020), fibroblasts and granulosa cells (Diem *et al.*, 2005), ovarian follicles (Gul *et al.*, 2009; Türedi *et al.*, 2016; Koohestani *et al.*, 2019; Azimipour *et al.*, 2020) fertilization and embryo development (Batellier *et al.*, 2008).

This review summarized available published studies that assessed the effects of exposing *in vitro* and *in vivo* produced mammalian embryos to cell phone radiation on subsequent embryo survival. Inconsistency of results might be due to different experimental settings, animal models or various effects of RF-EMR in animals of different sizes (Santini *et al.*, 2018). It appears that numerous factors could affect the outcome of RF-EMR experiments such as exposure conditions, cell phone types, frequency of

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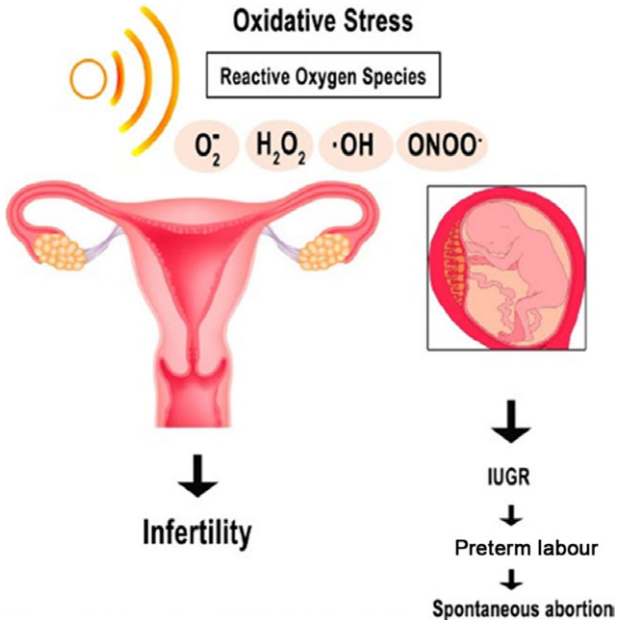

Figure 1. Effect of electromagnetic radiation on female reproduction and fetal development. IUGR, intrauterine growth restriction (Agarwal *et al.*, 2012).

the mobile phone, and duration of exposure (Safian *et al.*, 2016). Also, RF-EMR from cellular phones always varies according to the surrounding environment and signal conditions. In addition, the amount of exposure to RF-EMR depends on the size of the exposed target, whether the target is stationary or moving, or whether the target is in air or liquid and, if in liquid, the properties of the liquid should be considered (Suzuki *et al.*, 2017).

Based on the Safian study, although RF-EMR did not have significant adverse effects on mouse embryo development, they hypothesized that ROS production may have adverse effects on subsequent embryo survival (Safian *et al.*, 2016). Several other studies have demonstrated that the exposure of different cell types to a 50 Hz RF-EMR led to an increase in the intracellular ROS levels (Ayşe *et al.*, 2010; Mannerling *et al.*, 2010; Patruno *et al.*, 2015; Calcabrini *et al.*, 2017; Santini *et al.*, 2018). Suzuki and colleagues announced that, even though 60 min exposure time did not cause spermatozoa ageing in *in vitro* condition, *in vivo* exposure for longer periods of time could affect fertilization, and early embryonic development (Suzuki *et al.*, 2017).

Personal protective measures and future view

Mobile phones are commonly placed on the lap or in pockets (Swerdlow *et al.*, 2011), therefore exposing the genital area to RF-EMF. Germ cells are more susceptible to RF-EMF, as they are rapidly dividing through meiosis and mitosis. EMF induces modifications in cellular levels such as activation of voltage-gated calcium channels, formation of free radicals, protein misfolding and DNA damage (Altun *et al.*, 2018). In the general population, pregnant women are at specific risk of exposure to environmental RF-EMF because of their higher oxygen consumption and amniotic fluid-induced ROS production (Çiğ and Nazıroğlu, 2015) (Fig. 1). Individuals should reduce their rate of exposure to RF-EMF-emitting devices including mobile phones (Okechukwu, 2020) as electromagnetic waves enhanced the amount of oxygen free radicals in the body that led to disturbance of spermatogenesis process and variations in spermatozoa membrane, which resulted in changes in capacitation, acrosome reaction and therefore disorder in the process of spermatozoa binding to the oocyte and failure of normal fertilization both *in vivo* and *in vitro* (Fatehi *et al.*, 2018). In addition, exposure to RF may lead to a decline in ovarian follicle reservoirs at the start of the prepubertal time (Türedi *et al.*, 2016).

To reduce the contact with RF-EMR radiated by cell phones, people should avoid keeping their phones inside their pockets, also use of hand-free materials and gadgets and mobile covers limit direct expose of cell phones to the body (Okechukwu, 2020). Also, people do not appreciate that a cell phone is a small base station and usually underrate the importance of distance between the cell phone and the body (Cousin and Siegrist, 2010). In analyzing the relationship between the distance of cell phone antenna from the body and SAR, Hossain and colleagues reported that increasing the distance of cell phone from the body led to diminishing of both electric and magnetic field strength to the body, therefore reducing SAR values (Hossain *et al.*, 2015). Furthermore, it is known that the EMF decreases with distance and magnetic induction as calculated below:

$$B = k.\frac{1}{d^a}$$

where k is a constant dependent on the source, d is the distance to the source in metres, and α is commonly between 1 and 3, based on the nature of the source (Staebler, 2017).

Moreover, it is known that vitamins C and E and some natural supplements are helpful in protecting body against RF-EMR effects (Okechukwu, 2018, 2020). There have been limited studies that assessed the consequence of cellular phone electromagnetic radiation on ART such as IVF or ICSI (Suzuki *et al.*, 2017), therefore additional investigations are required to examine the influence of cell phone usage in ART.

Conclusion

At this time, it is difficult from the available animal studies to document confidently the role of RF-EMR exposure on human embryo development, both *in vivo* and *in vitro*. Further investigations with complementary techniques will be necessary to understand the mechanism of action of RF-EMR emitted by cell phones and the consequences on mammals, particularly human beings.

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

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