Bundesamt
für Strahlenschutz

**Spotlight on EMF Research**

# Spotlight on “Detrimental effects of electromagnetic radiation emitted from cell phone on embryomorphokinetics and blastocyst viability in mice” by Seify et al. in Zygote (2024)

## Category [radiofrequency, in vitro study]

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Competence Centre Electromagnetic Fields (KEMF)

## 1 Putting the paper into context by the BfS

Embryonic development is a critical stage of life known to be extremely sensitive to environmental influences [2]. Possible detrimental effects of exposure to radiofrequency electromagnetic fields (RF-EMF) on fertility, embryonic toxicity and birth outcomes have been investigated in animal and cell culture models in several experimental studies over the years; however, the results are inconsistent. A recently published systematic review on the effects of RF-EMF on pregnancy and birth outcomes by Cordelli and colleagues [3], that we have featured in a spotlight (http://nbn-resolving.de/urn:nbn:de:0221-2024061244261) did not find reliable evidence for detrimental effects. In the present study, the effects of RF-EMF exposure are tested on the earliest of embryonal stages that occur right after fertilization.

## 2 Results and conclusions from the authors‘ perspective

According to the authors, there are many studies showing detrimental effects of RF-EMF exposure on different parameters of male and female fertility, including the development of embryos and foetuses. In this study, the authors aimed to assess the effects of cell phone radiation on preimplantation embryo morphokinetics and blastocyst (five to six days old embryos) viability of mice *in vitro*.

To harvest a reasonable number of zygotes, n = 20 female NMRI mice (6–8 weeks old) were treated with hormones to induce superovulation (production of a larger than usual number of eggs) before being mated with male mice. After successful mating the mice were sacrificed, the ovaries removed and overall n = 300 zygotes extracted, which were then assigned to either a control group (n = 150) or an exposure group (n = 150). RF-EMF exposure was performed using a commercial cell phone with a specific absorption rate (SAR) of 0.683 – 0.725 W/kg and a frequency range of 900 – 1800 MHz. During exposure, the cell phone remained continuously in talk mode. The embryos of the exposed group were exposed to RF-EMF for 30 min

on day 1 in an incubator, cultured for up to 4 subsequent days and fixed in paraffin. The control group was treated equally, except for the exposure step being omitted. The developmental progress of the embryos, including morphokinetic aspects, was monitored daily using time-lapse microscopy, where the embryos were manually examined to assess various parameters of early cleavage kinetics. To assess cell viability, the blastocysts were stained with Hoechst and propidium iodide (PI) and analysed under a fluorescence microscope. To determine embryo quality, they were graded according to a reported grading system [4] from A-D (A and B: high-quality embryos, C and D: low-quality embryos) in terms of fragmentation and granularity.

The analysis of blastocyst cell viability showed a significantly increased number of dead cells and a decreased number of living cells in the exposed group in comparison to the control group. In the investigation of morphokinetics, the exposed group showed a significantly longer division time in comparison to the control group on days 2, 8, 10 and 12. Also in terms of the blastocoel formation (indicating the transition from the morula stage to the blastula stage), there was a significant delay in the exposed group in comparison to the control group. Next, cleavage abnormalities were investigated. Here, the exposed group showed significantly increased rates of fragmentation, reverse cleavage (failed cytokinesis), vacuole formation, and embryo arrest. Considering the number of embryos in different life stages, no differences were observed in the zygote, two-cell and four-cell stages, but starting from the eight-cell stage, the exposed group showed significantly lower embryo numbers up to the hatched blastocyst stage.

According to the authors, the results of the study suggest that exposure to RF-EMF exposure can lead to embryonic defects in the early stages after fertilization. Since the observations made appeared to have time-dependent characteristics, the authors claim that this is due to non-thermal effects of RF-EMF and refer to the results of another study [5]. Mechanistically, the authors assume that RF-EMF could have increased the amounts of reactive oxygen species (ROS), which has detrimental effects for embryonal development. The authors recommend further studies to obtain indisputable results.

# 3 Comments by the BfS

If low-intensity RF-EMF indeed had an adverse effect on the early stages of embryonic development *in vitro*, this would be a relevant discovery requiring further investigation. However, the data and methodology presented in the work by Seify et al. do not allow drawing such a conclusion.

While the authors used a fairly large number of cells per group (n = 150) from different animals and employed standard and reliable methods for identifying dead cells or measuring real-time cellular dynamics, the missing blinding and randomization steps and the lack of a benchmark with a positive control substantially reduce the reliability of the published results. It is even unclear whether there was a sufficient RF-EMF exposure contrast between the experimental groups at all and, if so, to what extent the cells were actually exposed. Using mobile phones in talk mode as exposure source is not recommended, as their transmission power is controlled by modern mobile networks, leading to unknown but typically much (orders of magnitude) lower SAR values than the maximum values specified in data sheets. Furthermore, the datasheet values are obtained in close vicinity of body phantoms and do not reflect the situation in the exposure setup with significant distance between the phone and the cell dishes. Hence, any independent replication of the results is impossible (because the exposure level remains unknown) und due to the presumably very low exposure contrast, it appears unlikely that the observed changes can indeed be caused by RF-EMF exposure.

Most reported differences between the groups appear to be small and, without appropriate positive control data, the clinical relevance of such changes is unclear. For instance, the absolute time differences between the two groups concerning cleavage divisions are roughly 2 to 5 hours at the late time points (t8 - t12) and therefore within the standard deviation of the controls. In other studies, it was demonstrated that blastocyst formation in mice normally happens between 3 and 3.5 days after conception [6], demonstrating that there is a natural range for this step that greatly exceeds the changes that are reported in the study. Also, the difference in numbers of living cells is small compared to the standard deviation of the control group.

However, the number of dead cells in the exposed group is twice as large as the one in the control group, and the analysis of malformation rates shows a statistically significant increase in the exposed group for four out of five malformations investigated. These malformations are severe defects, particularly the embryo arrest, which mostly happens in case an embryo has chromosomal aberrations [7], which at this early stage usually originate from damaged gametes. However, given the methodological limitations mentioned above and the fact that no sham exposure procedure has been applied to the control group, the study of Seify et al. provides no reliable evidence that there is a causal relation between the observed differences and RF-EMF exposure.

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