

# GSM 900 MHz microwave radiation affects embryo development of Japanese quails

Olexandr Tsybulin<sup>1</sup>, Evgeniy Sidorik<sup>2</sup>, Sergiy Kyrylenko<sup>3</sup>, Diane Henshel<sup>4</sup> & Igor Yakymenko<sup>1,2</sup>

<sup>1</sup>Department of Biophysics, Bila Tserkva National Agrarian University, Bila Tserkva, Ukraine, <sup>2</sup>R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of NAS of Ukraine, Kyiv, Ukraine, <sup>3</sup>Department of Biology, Masaryk University, Brno, Czech Republic, and <sup>4</sup>School of Public and Environmental Affairs, Indiana University Bloomington, Bloomington, Indiana, USA

A wide range of non thermal biological effects of microwave radiation (MW) was revealed during the last decades. A number of reports showed evident hazardous effects of MW on embryo development in chicken. In this study, we aimed at elucidating the effects of MW emitted by a commercial model of GSM 900 MHz cell phone on embryo development in quails (*Coturnix coturnix japonica*) during both short and prolonged exposure. For that, fresh fertilized eggs were irradiated during the first 38 h or 14 days of incubation by a cell phone in “connecting” mode activated continuously through a computer system. Maximum intensity of incident radiation on the egg’s surface was  $0.2 \mu\text{W}/\text{cm}^2$ . The irradiation led to a significant ( $p < 0.001$ ) increase in numbers of differentiated somites in 38-hour exposed embryos and to a significant ( $p < 0.05$ ) increase in total survival of embryos from exposed eggs after 14 days exposure. We hypothesized that observed facilitating effect was due to enhancement of metabolism in exposed embryos provoked via peroxidation mechanisms. Indeed, a level of thiobarbituric acid (TBA) reactive substances was significantly ( $p < 0.05$ – $0.001$ ) higher in brains and livers of hatchlings from exposed embryos.

Thus, observed effects of radiation from commercial GSM 900 MHz cell phone on developing quail embryos signify a possibility for non-thermal impact of MW on embryogenesis. We suggest that the facilitating effect of low doses of irradiation on embryo development can be explained by a hormesis effect induced by reactive oxygen species (ROS). Future studies need to be done to clarify this assumption.

**Keywords** Electromagnetic radiation, Radiofrequency, Cell phone, Non thermal effect, Embryogenesis, Somitogenesis, Peroxidation

## INTRODUCTION

During the last few decades, worldwide intensive implementation of GSM mobile communication systems has occurred. This has led to a dramatic increase in levels of radiofrequency/microwave (RF/MW) irradiation of the human population and environment. Notably, general public safety limits for electromagnetic fields (EMF) proposed by the International Commission on Non-Ionizing Radiation Protection

---

Address correspondence to Igor Yakymenko, Department of Biophysics, Bila Tserkva National Agrarian University, 8 Soborna Pl, Bila Tserkva, 09117 Ukraine. E-mail: yakymenko@btsau.net.ua

(ICNIRP, 1998), and accepted by mobile communication industry, have been elaborated taking into account solely acute thermal effects of radiation. Particularly, ICNIRP directly states that “guidelines are based on short-term, immediate health effects such as stimulation of peripheral nerves and muscles, shocks and burns caused by touching conducting objects, and elevated tissue temperatures resulting from absorption of energy during exposure to EMF”. Obviously, applicability of such an approach could be questioned for cases dealing with long-term exposure, i.e., many-year irradiation of human brain during chronic cell phone use, etc. In fact, appreciable non thermal effects of MW have been detected in different experimental models. These effects include substantial metabolic changes (Volkow et al., 2011) as well as significant changes in expression of heat shock proteins (Czyz et al., 2004; De Pomerai et al., 2000; Weisbrot et al., 2003), oxidative stress (Agarwal et al., 2009; Ozguner et al., 2005; Ozgur et al., 2010; Tkalec et al., 2007), DNA damage (De Iuliis et al., 2009; Lai and Singh, 1997; Sarkar et al., 1994), autoimmune pathology (Grigoriev et al., 2010; Ivanov et al., 2010; Vinogradov and Dumansky, 1974), and carcinogenesis (Chou et al., 1992; Repacholi et al., 1997; Szmigielski et al., 1982; Yang et al., 2010).

Additionally, recent epidemiological studies raised concerns about potential hazards of long-term MW exposure for human health. Thus, a significantly increased risk of development of certain tumors, particularly brain tumors (Hardell et al., 2005, 2006, 2007) and parotid gland tumors (Auvinen et al., 2002; Sadetzki et al., 2008), have been detected in long-term or “heavy” users of cell phones. Likewise, increased risk of carcinogenesis (Eger et al., 2004; Wolf and Wolf, 2007), as well as non cancerous physiological changes (Abdel-Rassoul et al., 2007; Santini et al., 2002) have been detected among inhabitants of areas located nearby transmitting base stations for mobile communication.

Due to increased public concerns, the intensive studies on non thermal effects of MW are nowadays carried out using various experimental models ranging from cell culture to whole animal models. We suggest that one of the most useful experimental models for studying the biological effects of MW could be developing a bird embryo. First of all, developing an embryo is very sensitive to the external factors and therefore is served as a classical model for risk assessment experiments (Henshel et al., 2003). The apparent advantage of this model is the fact that development of a bird embryo happens independently of a mother organism when artificial incubation devices are employed. In addition, an incubation period of certain laboratory bird embryos, such as Japanese quails, is 17 days which is short enough for efficient experimentation yet still sufficient for manifestation of irradiation effects.

Recently, significant hazard effects of low intensity MW on embryo development and survival of chicken and quails have been described (Batellier et al., 2008; Grigoriev, 2003; Ingole and Ghosh, 2006; Inouye et al., 1982; Saito et al., 1991). On the other hand, earlier reports on MW irradiation of quail embryos did not reveal significant non thermal effects (Byman et al., 1985; Galvin et al., 1980; Gildersleeve et al., 1987). Therefore, to clarify the apparent controversy, we aimed at assessment of biological effects of MW emitted by commercial cell phone using developing bird embryo as a model. The quail eggs were irradiated using commercial model of GSM 900 MHz cell phone which emitted low-intensity MW in compliance with the most tough national safety standards implemented in developed countries. We studied the effects of MW on somitogenesis after short-term exposure and the survival rate of embryos and hatchability after relatively long-term exposure, and found that the exposure facilitates development of embryos under both short and long term exposure conditions. We hypothesized that such developmental enhancement could occur due to increased metabolism stimulated (e.g., via glucose uptake) by reactive oxygen species induced by irradiation. We therefore found that the level of lipid

peroxidation was indeed increased in various tissues of hatchlings after MW exposure of embryos in ovo. Taken together, we show that cell phone irradiation induces significant non thermal effects on both developmental somitogenesis and total survival rate of quail embryos.

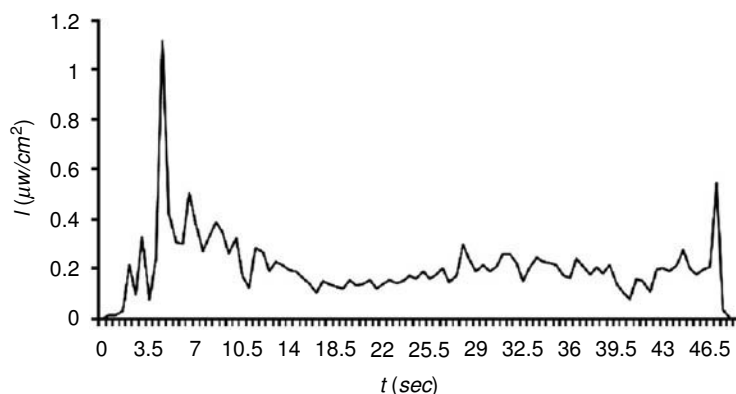
## MATERIALS AND METHODS

### Biological Material

The fresh fertilized quail eggs from commercial poultry farm (Bila Tserkva, Ukraine) were used. The eggs were selected according to the incubation standards (11–12 g, clean, correct shape, and pigmentation, without shell cracks). For each experiment two similarly selected groups of eggs were formed, from which one group was exposed while another group was used as an unexposed control. The incubation of eggs was performed in a laboratory incubator (920 mm × 590 mm × 530 mm) with the possibility of automatic rotation of incubation trays (ILU-F-0.3, Russia). Metal covers were removed from the door and sides of the incubator and replaced by plastic covers to avoid shielding or reflection of MW used for irradiation. Incubation conditions were optimal for quail eggs: temperature of incubation during the first 14 days was  $38.3 \pm 0.2$  °C, relative humidity 60% (Yakimenko et al., 2002). We used horizontal trays for incubation and manual rotation of eggs three times per day. After 14 days of incubation the temperature of incubation was lowered to  $37.5 \pm 0.2$  °C and eggs were incubated for additional 3 days without rotating them over.

### Source of MW Radiation

A commercial model of a cell phone GSM 900 MHz (Nokia 3120) operated by the Ukrainian national operator of mobile communication (Kyivstar) was used as a source of MW radiation. RF Field Strength Meter (Alfalab Inc, Salt Lake City, Utah, USA) was used for measurement of intensity (power density) of MW radiation. An average intensity of MW radiation at 3 cm distance from the cell phone was  $0.21 \pm 0.014$   $\mu\text{W}/\text{cm}^2$  during “connecting” (Fig. 1); an average intensity of radiation at 10 cm from the cell phone was  $0.024 \pm 0.003$   $\mu\text{W}/\text{cm}^2$ . Specific Absorption Rate (SAR) of this particular cell phone model is 0.79 W/kg according to its manual. During the auto-redial (without pressing the “answer” button and going into a talking mode), the phone emitted non modulated MW radiation of carrier frequency 890–915 MHz with channel rotation frequency 217 Hz (Hyland, 2000).



**FIGURE 1** A typical plot of intensity of GSM 900 microwaves emitted from a commercial cell phone (Nokia 3120) in “connecting” mode during one call: *t* – time in seconds; *I* – measured intensity of MW at 3 cm from a cell phone.

### Incubation and Irradiation

We studied biological effects of both relatively short-term (38 h, very early embryo) and long-term (14 days) irradiation. The cell phone was placed inside the incubator on a plastic stand 3 cm from the surface of eggs (Fig. 2). Eggs were placed horizontally by long axis. Irradiation of exposed groups of embryos was performed continuously by the muted cell phone in “connecting” mode using auto-redial from a computer program AutoRingUp ([www.autoringup.ru](http://www.autoringup.ru), Russia). The program ensured redial of the cell phone number immediately after disconnection from a previous call. Each connection attempt lasted about 45 s. The exposed and control groups of eggs were incubated simultaneously in the same incubator camera separated by 6 cm and shielded by aluminum foil from each other. Temperature at the surface of incubation eggs in both control and exposed groups was controlled during all period of incubation by sensitive thermometers with 0.1°C precision.

For the effects of short-term irradiation on early embryo development we observed the embryos microscopically (see below). One of the most objective integral index of early embryo development of birds is a number of differentiated somites (Hamburger and Hamilton, 1992). Therefore, we estimated a number of pairs of differentiated somites in exposed and control embryos after 38 h of incubation/irradiation. Possible developmental abnormalities were also assessed. In total, irradiation of eggs during 38 h of incubation comprised of ca. 3,000 calls. For each experiment the exposed and control groups of fertilized eggs ( $n = 10$ ) were formed.

Total survival and hatchability of quail embryos receiving relatively long exposure was also assessed. For that, exposed and control groups of fertilized eggs ( $n = 56-68$ ) were irradiated continuously during the first 14 days of incubation. Distance from the cell phone to surface of the eggs in these experiments varied from 3–10 cm. Irradiation of eggs comprised of ca. 26,900 calls in total for this experiment.

### Analysis of Somitogenesis

Analysis of somitogenesis was carried out as described by Yakymenko et al. (2011). Briefly, after 38 h of incubation embryo development was stopped by cooling the eggs in cold water (10°C). After shell breaking and removal of the whites, embryos were taken off from the surface of yolk using filter paper rings. Embryos were then washed in cold PBS and fixed by 4% formaldehyde. Calculation of numbers of differentiated somites and visual analysis of abnormalities of development of embryos were carried out under light microscope with 24x magnification.



**FIGURE 2** Irradiation of quail embryos in ovo (exposed group) to MWs of commercial GSM 900 MHz cell phone in a plastic incubation tray within the incubator.

### Analysis of Embryo Survival and Hatchability

Analysis of embryo survival and hatchability was done after the end of incubation and hatching as described (Yakimenko et al., 2002). The eggs of exposed and control groups were separated in hatching trays before the hatching, and hatchlings of each group were collected separately. The wastes of incubation were analyzed according to the poultry standards for Japanese quails. For that, the eggs that failed to hatch were opened for macroscopical observation. Wastes were classified according to the time of embryonic mortality (early stages: up to the 5th day of development; middle stages: from 6th–14th days of development; and final stages: from 15th–17th days). In addition, non fertilized eggs were identified in each group and excluded from follow-up analysis.

### Lipid Peroxidation Levels

Level of lipid peroxidation in the tissues of the brain, liver, and heart of hatchlings was assessed in reaction with thiobarbituric acid (TBA) in the present of  $\text{Fe}^{2+}$  (Andreeva et al., 1988; Draper and Hadley, 1990). Briefly, after decapitation of hatchlings from exposed and control groups ( $n = 10$ ) the brains, livers, and hearts were extracted, frozen, and then homogenized separately for each chick. Homogenates were diluted 1:50 in 50 mM Tris-HCl, pH 7.6 buffer. To 0.15 ml of the diluted homogenate 1.5 ml of 1% orthophosphoric acid was added followed by addition of 0.5 ml 0.75% of TBA, and  $\text{FeSO}_4 \times 7\text{H}_2\text{O}$  to 0.5  $\mu\text{mole/l}$ . The reaction was carried out for 30 min in test tubes placed in a boiling water bath and stopped in cold water. Then the test tubes were centrifuged at 3,000 g for 10 min. The level of TBA reactive substances was measured in supernatants using a spectrophotometer (Specoll 11, Germany) at 532 nm. The level of malondialdehyde (MDA) in samples was calculated in nmole per g of wet tissues using absorbance coefficient  $E = 1.56 \cdot 10^5 \text{ mole}^{-1} \text{ cm}^{-1}$ .

### Statistical Analysis

Student's t-test and Chi square test were used for the statistical analysis, with a significance levels \*  $p < 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$  as compared with the matched controls.

## RESULTS

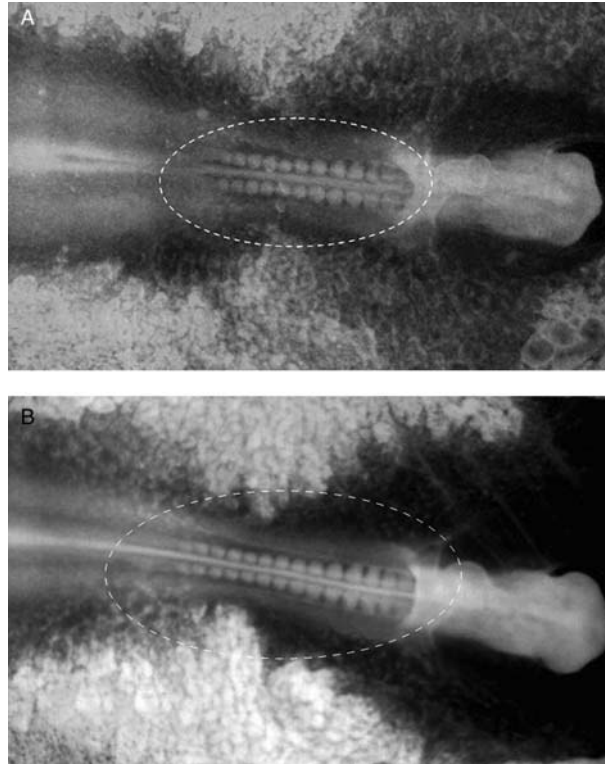
### The number of differentiated somites increased in exposed early-stage embryos

The low-intensity microwave irradiation of quail embryos in ovo during 38 h of incubation by GSM 900 MHz commercial cell phone in connecting mode significantly ( $p < 0.001$ ) increased the number of differentiated somites after 38 hours of incubation. The exposed embryos developed  $13.11 \pm 0.30$  pairs of differentiated somites while in the control groups  $11.41 \pm 0.29$  pairs of differentiated somites were detected (Fig. 3). Microscopic analysis did not reveal any developmental abnormalities either in control, or in the exposed groups of embryos. Measurement of temperature at the surface of incubating eggs did not show any differences between exposed and control groups (not shown). Thus, irradiation emitted by commercial cell phone can affect early embryonic development.

### Irradiation increased survival rate and hatchability

Experiments on microwave irradiation of quail embryos in ovo during the first 14 days of incubation revealed a significant ( $p < 0.05$ ) increase in total survival rate of quail embryos (Table 1). Thus, the hatchability was increased by the 13.3 absolute percent (or by 18.7 relative percent) as compared with the control. The hatching



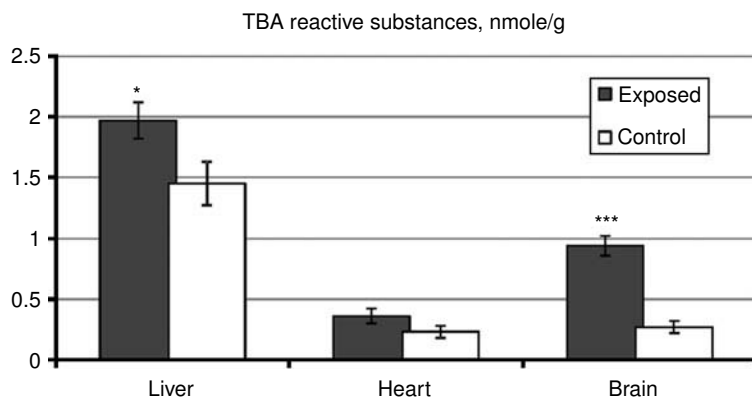


**FIGURE 3** Microscopic pictures ( $\times 24$ ) of 38-h quail embryos (ventral): A - control (11 pairs of somites); B - after MWs irradiation during 38 hour of incubation in “connecting” mode by a cell phone with MW intensity of  $0.21 \pm 0.014 \mu\text{W}/\text{cm}^2$  (14 pairs of somites).

occurred synchronously inside each group and took place 3–4 h earlier in exposed groups as compared with the controls. The hatchlings from the control and exposed groups were normally developed without any detectable exterior abnormalities. The body mass of hatchlings was similar in both exposed and control groups ( $7.11 \pm 0.21$  g and  $7.1 \pm 0.15$  g, respectively). Taken together, this data suggest that irradiation emitted by commercial cell phones can induce routinely observable effects on both early and late stages of embryonic development.

TABLE 1 GSM 900 MHz microwaves emitted from commercial cell phone increases embryo survival of Japanese quail. Eggs of exposed group were irradiated constantly during the first 14 days of incubation inside an incubation chamber by a cell phone in “connecting” mode placed at 3–10 cm over the egg’s surface; intensity of radiation on egg’s surface was from  $0.024 \pm 0.003$  to  $0.21 \pm 0.014 \mu\text{W}/\text{cm}^2$ ; control group of eggs was shielded from exposure by aluminium foil at 6 cm from exposed group and incubated in the same incubation chamber.

Indices	Control group		Exposed group	
	number	percent	number	percent
Number of fertilized eggs	114	100	115	100
Lost embryos				
at 1–5 days	14	12,28	7	6,09
at 6–15 days	17	14,91	10	8,70
at 16–17 days	2	1,75	1	0,87
Hatched	81	71,05	97	84,35*



**FIGURE 4** Irradiation of GSM 900 MHz microwaves emitted by a commercial cell phone induce increased levels of TBA reactive substances in tissues of Japanese quail hatchlings. Incubating eggs were irradiated during the first 14 days of incubation inside an incubation chamber, in “connecting” mode at 3–10 cm distance from cell phone, intensity of radiation on eggs surface was from  $0,024 \pm 0,003$  to  $0,21 \pm 0,014 \mu\text{W}/\text{cm}^2$ , control group of eggs was shielded from exposure by aluminium foil at 6 cm from exposed group and incubated in the same incubation chamber ( $n = 10$ ; mean  $\pm$  SME is shown).

#### Irradiation resulted in increased levels of lipid peroxidation in various tissues of quail hatchlings

To study whether observed biological effect of irradiation could be explained by peroxidation mechanisms, the brain, heart, and liver tissues from one-day old hatchlings were subjected to measurement of lipid peroxidation by TBA assay. We found, that indeed the tissues in one-day chicks from exposed eggs had higher levels of TBA reactive substances (Fig. 4). Basal level of lipid peroxidation was highest in liver and showed significant increase upon irradiation. Especially conspicuous increase was detected in brains, where the levels were higher by 3.5 folds in comparison with the unexposed controls ( $p < 0.001$ ). The basal level of TBA reactive substances was lowest in hearts; it also showed a trend to increase upon irradiation but did not reach statistical significance. Therefore, irradiation of developing embryos by commercial cell phones leads to substantial increase in lipid peroxidation, especially in brains of affected chicks.

#### DISCUSSION

Developing bird embryos can serve as a sensitive, convenient, and useful model for assessment of a wide range of risk factors. Nevertheless, only a few studies have so far been carried out on non thermal effects of RF/MW using this model (Batellier et al., 2008; Grigoriev, 2003; Ingole and Ghosh, 2006; Saito et al., 1991; Yakymenko et al., 2011; Zareen et al., 2009). Remarkably, majority of this studies resulted in alarming conclusions. In a number of such reports the irradiation of embryos during the whole period of incubation was used. These studies demonstrated adverse effects of irradiation on embryo development with significant increase of embryonic mortality. Thus, the irradiation of chicken embryos by 428 MHz RF radiation with  $5.5 \text{ mW}/\text{cm}^2$  power density led to increase in total embryo mortality from 15.8% in control to 60.0% in exposed group (Saito et al., 1991). In another report, irradiation of chicken embryos by cell phone MW of GSM standard (1,714 MHz, electric field strength 2–16 V/m, repeated calls during 1.5 min and rest during 0.5 min for 21 days of incubation) resulted in total mortality of embryos of 75% as compared to 16% of mortality in control (Grigoriev, 2003). Moreover, use of SAGEM (900 MHz) cell

phone in call position for irradiation of chicken embryos (electric field strength from 0.31 – 15.4 V/m) during the whole period of incubation gave a rise to 31.2% of embryo mortality in exposed groups as compared to 15.3% in sham-exposed groups (Batellier et al., 2008).

In the present study, we used a commercial cell phone of GSM 900 MHz standard emitting low intensity MW (up to 0.21  $\mu\text{W}/\text{cm}^2$ ), which corresponded to the strictest national standards of some countries (as an example, the Ukrainian Ministry of Health recommended safety limit of RF/MW intensity for general public to not exceed 2.5  $\mu\text{W}/\text{cm}^2$  compared with ICNIRP recommended limits of about 450  $\mu\text{W}/\text{cm}^2$  in 900 MHz range). Therefore, we in fact operated with say “extremely low intensity” of MW.

It is generally accepted that the number of differentiated somites during the first days of incubation is the most objective index of stage of bird embryo development (Hamburger and Hamilton, 1992). Recently, we observed increased of morphological malformations in somitogenesis and slight retardation of development in quail embryos after 20-h exposure in ovo to GSM 850 MHz with to 15  $\mu\text{W}/\text{cm}^2$  intensity (Yakymenko et al., 2011). Importantly, the intensity of MW used in the present study was 2–3 orders of magnitude lower. Nevertheless, we were able to detect substantial biological effects of such low-intensity irradiation. Interestingly, the embryos exposed to such extremely low intensity of MW showed substantially increased development at early stages as compared with controls. In addition, no developmental abnormalities were observed. According to the previously published data, facilitation of embryo development after the WM exposure from stage with 11–12 to stage with 13–14 pairs of differentiated somites corresponds to 3–4 h of acceleration of embryo development (Hamburger and Hamilton, 1992). It is of note here that in our experiments, when embryos were exposed to MW during 14 days, we also observed that hatching occurred 3–4 h earlier than the control. This therefore suggests that the irradiation of embryo only during early development results in the same enhancement of hatching as compared to prolonged irradiation during later phases. Importantly, this might suggest that the early embryo shows increased sensitivity to irradiation.

Remarkably, the very low intensity of irradiation and routine temperature control at surface of incubating eggs suggest that a chance that observed biological effects occurred via thermal mechanisms can be ruled out. In this connection we may draw an analogy to our previous findings which indicated high sensitivity of early stage quail embryo to low intensity monochromatic radiation of visible range (Tsybulin and Yakymenko, 2009; Yakymenko et al., 2002), in which thermal effects were considered negligible. To that, the intensity of electromagnetic radiation in present study was by 3 orders magnitude lower.

In this study, we showed an increased embryo survival and hatchability in exposed groups of eggs. The survival rate of exposed embryos was higher during all periods of incubation as compared with the control. These findings may contradict to the previous studies on MW irradiated chicken eggs during the incubation (Batellier et al., 2008; Grigoriev, 2003; Saito et al., 1991). However, we suggest that these data can in fact be considered complimentary. Indeed, in the present study we used substantially lower intensity of MW than in other reports. Thus, the Saito et al. (1991) study used MW intensity higher by 4 orders of magnitude. Consequently, with such low intensity as was used in our study, an absence of any observable biological effects on embryogenesis could be expected. Nevertheless, we observed significant effects on embryo development. Taking into account a large amount of data about adverse effects of MW including hazard effects on embryogenesis (extensively reviewed in, e.g., Blank and Goodman, 2009; Yakymenko and Sidorik, 2010), our findings most probably demonstrate effect of hormesis by low doses of MW irradiation.



It is known that low doses of a potentially harmful factor can lead to a stimulatory effect in exposed biological systems (Stebbing, 1982). In particular, low doses of ionizing radiation reveal apparent stimulatory effects in certain biological experiments (Calabrese and Baldwin, 2000). Certainly, an important task would be to uncover the internal mechanism of such stimulation. In a simplified explanation, it can be considered as a result of activation of cellular stress response pathways in response to action of a low intensity stress factor (Mattson, 2008; Ristow and Zarse, 2010).

It is noteworthy here that certain epidemiological studies detected a decreased risk of tumor development among particular categories of cell phone users. One of the most renowned reports of this series is the Interphone study, in which a decreased risk of two brain tumors among cell phone users was explained by certain methodological limitation of the study protocols (Cardis et al., 2010). In this respect, we can suggest that these results can in fact also reflect hormesis effect of MW for “light” users of cell phones, which would be changed towards adverse effects with an increase of total dose of irradiation. Indeed, for “heavy” users of cell phones (1,640 h of talks over cell phone during 4 years) the same study indicated an increase of risk for meningioma by 4.8 folds and for glioma by 3.77 folds as compared to matched controls.

To elucidate the biochemical mechanisms of effects of irradiation observed in our study, it is important to note that a significant increase of TBA reactive substances in tissues of quail hatchlings from exposed embryos has been detected. This indicates particular appreciable changes in embryonal metabolism under the low intensity MW irradiation. Consequently, it points to the increased lipid peroxidation of hatchling’s tissues from exposed embryos, which is closely connected to levels of reactive oxygen species (ROS). Importance of detailed mechanisms of such changes warrants further studies. In addition to well-known adverse effects of increased level of ROS in cells, it is also an intrinsic feature of living cells which plays an important role in growing tissues in particular (Kamata and Hirata, 1999; Valko et al., 2007). Moreover, we previously detected a significant increase of lipid peroxide level in liver of intensively growing quail embryos after red light stimulation leading to no adverse effects (Iakymenko, 2001). However, a dramatic increase of lipid peroxidation in brain tissues as compared to other tissues in the present study draws particular attention. One of the reasons of this can be that the brain of an embryo is the most moisture organ, which means that it absorbs MW most intensively as compared to other tissues (de Salles et al., 2006). Recently, an increase of ROS level as a result of MW exposure has been detected in different biological models including cell culture (Friedman et al., 2007), human spermatozoa (Agarwal et al., 2009), and organism of rodents (Ozguner et al., 2005; Ozgur et al., 2010). In addition, activation of ROS production in mitochondria (De Iuliis et al., 2009) as well as activation of cell membrane HADH-oxidase system (Friedman et al., 2007) in response to MW was demonstrated. However, in all these studies the level of MW intensity was substantially higher than in our study.

Moreover, cellular ROS are increasingly recognized as cellular signaling molecules, which regulate cellular metabolism including, e.g., glucose uptake (Leloup et al., 2006; Merry et al.; Nemoto et al., 2000; Ristow and Zarse, 2010). Taking this into account, our results fall in agreement with recently published observation showing that 50-min cell phone exposure was associated with increased brain glucose metabolism in the region closest to the antenna (Volkow et al., 2011). This might suggest that glucose metabolism in exposed embryos was induced via mechanism dependent on observed increase in lipid peroxidation, which resulted in more intensive embryo development.

It is also of note that in our experiments the intensity of MW irradiation ranged from  $0.024 \pm 0.003$  to  $0.21 \pm 0.014 \mu\text{W}/\text{cm}^2$  (depending on the distance to the phone antenna) which can add to the variability in magnitudes of observed effects. This should be taken into account and controlled in future experiments. On the other hand, in Batellier et al.'s (2008) study, MW variations in strength of electric field from 0.31–15.4 V/m only slightly changed adverse effect on embryo development (Batellier et al., 2008).

Taken together, our findings point to potentially high biological activity of MW of extremely low intensity, which is substantially lower than safety limits for RF/MW currently accepted by international regulating bodies. The appreciable effect of low intensity MW of GSM 900 MHz on quail embryo development is important as an indicator of non-thermal biological effects of MW regardless of a direction of influence being stimulatory or adverse. Further comprehensive studies need to be done especially in connection with practically unlimited irradiation of general public over the world by low intensity but chronic exposure of MW both from personal cell phones and base transmitting stations for mobile communication. As to a possible importance of such research specifically for human embryo development, a large-scale epidemiological study which showed a statistically significant increase of behavioral problems for children with prenatal (and postnatal) exposure to cell phone radiation (Divan et al., 2008) could be noted.

In conclusion, our data confirm biological activity of low-intensity microwave radiation emitted by commercial cell phones of GSM 900 MHz standard. Continuous 900 MHz irradiation from connecting cell phone with average intensity of MW up to  $0.21 \mu\text{W}/\text{cm}^2$  significantly stimulated early development and total survival rate of quail embryos. Observed effects could be explained by increased levels of lipid peroxidation in brains and livers of hatchlings from MW exposed embryos as compared to controls. These data warrant further research for assessment of a dose-effect dependence of MW influence on embryogenesis and interpretation of obtained results. In general, our findings emphasize the need for taking into account the non thermal effects in reassessment of safety limits for non ionizing electromagnetic radiation.

## ACKNOWLEDGEMENTS

This study was supported by National Academy of Sciences of Ukraine (grant No 2.2.5.349), received a financial contribution from the European Community within the Seventh Framework Programme (FP/2007-2013) under Grant Agreement No. 229603, and was also co-financed by the South Moravian Region via SoMoPro programme.

## Declaration of interest

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

## REFERENCES

- Abdel-Rassoul, G., El-Fateh, O. A., Salem, M. A., et al. (2007). Neurobehavioral effects among inhabitants around mobile phone base stations. *Neurotoxicology* 28(2):434–440.
- Agarwal, A., Desai, N. R., Makker, K., et al. (2009). Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study. *Fertil. Steril.* 92(4):1318–1325.
- Andreeva, L., Kogemiakin, L., Kishkun, A. (1988). Modification of lipid peroxidation evaluation method according to the reaction with thiobarbituric acid. *Laboratornoe delo*(11):41–43.

- Auvinen, A., Hietanen, M., Luukkonen, R., Koskela, R. S. (2002). Brain tumors and salivary gland cancers among cellular telephone users. *Epidemiology* 13(3):356–359.
- Batellier, F., Couty, I., Picard, D., Brillard, J. P. (2008). Effects of exposing chicken eggs to a cell phone in “call” position over the entire incubation period. *Theriogenology* 69(6):737–745.
- Blank, M., Goodman, R. (2009). Electromagnetic fields stress living cells. *Pathophysiology* 16(2–3):71–78.
- Byman, D., Battista, S. P., Wasserman, F. E., Kunz, T. H. (1985). Effect of microwave irradiation (2.45 GHz, CW) on egg weight loss, egg hatchability, and hatchling growth of the Coturnix quail. *Bioelectromagnetics* 6(3):271–282.
- Calabrese, E. J., Baldwin, L. A. (2000). Radiation hormesis: its historical foundations as a biological hypothesis. *Hum. Exp. Toxicol.* 19(1):41–75.
- Cardis, E., Deltour, I., Vrijheid, M., et al. (2010). Brain tumour risk in relation to mobile telephone use: results of the INTERPHONE international case-control study. *Int. J. Epidemiol.* 39(3):675–694.
- Chou, C. K., Guy, A. W., Kunz, L. L., et al. (1992). Long-term, low-level microwave irradiation of rats. *Bioelectromagnetics* 13(6):469–496.
- Czyz, J., Guan, K., Zeng, Q., et al. (2004). High frequency electromagnetic fields (GSM signals) affect gene expression levels in tumor suppressor p53-deficient embryonic stem cells. *Bioelectromagnetics* 25(4):296–307.
- De Iuliis, G. N., Newey, R. J., King, B. V., Aitken, R. J. (2009). Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. *PLoS One* 4(7):e6446.
- De Pomerai, D., Daniells, C., David, H., et al. (2000). Non-thermal heat-shock response to microwaves. *Nature* 405(6785):417–418.
- de Salles, A. A., Bulla, G., Rodriguez, C. E. (2006). Electromagnetic absorption in the head of adults and children due to mobile phone operation close to the head. *Electromagn. Biol. Med.* 25(4):349–360.
- Divan, H. A., Kheifets, L., Obel, C., Olsen, J. (2008). Prenatal and postnatal exposure to cell phone use and behavioral problems in children. *Epidemiology* 19(4):523–529.
- Draper, H. H., Hadley, M. (1990). Malondialdehyde determination as index of lipid peroxidation. *Meth. Enzymol.* 186:421–431.
- Eger, H., Hagen, K., Lucas, B., et al. (2004). Einfluss der räumlichen Nähe von Mobilfunksendeanlagen auf die Krebsinzidenz. *Umwelt-Medizin-Gesellschaft* 17:273–356.
- Friedman, J., Kraus, S., Hauptman, Y., et al. (2007). Mechanism of short-term ERK activation by electromagnetic fields at mobile phone frequencies. *Biochem. J.* 405(3):559–568.
- Galvin, M. J., McRee, D. I., Lieberman, M. (1980). Effects of 2.45-GHz microwave radiation on embryonic quail hearts. *Bioelectromagnetics* 1(4):389–396.
- Gildersleeve, R. P., Galvin, M. J., McRee, D. I., et al. (1987). Reproduction of Japanese quail after microwave irradiation (2.45 GHz CW) during embryogeny. *Bioelectromagnetics* 8(1):9–21.
- Grigoriev, Y. G. (2003). Biological effects of mobile phone electromagnetic field on chicken embryos (risk assessment using the mortality rate). *Radiats. Biol. Radioecol.* 43(5):541–543.
- Grigoriev, Y. G., Grigoriev, O. A., Ivanov, A. A., et al. (2010). Confirmation studies of Soviet research on immunological effects of microwaves: Russian immunology results. *Bioelectromagnetics* 31(8):589–602.
- Hamburger, V., Hamilton, H. L. (1992). A series of normal stages in the development of the chick embryo. 1951. *Dev. Dyn.* 195(4):231–272.
- Hardell, L., Carlberg, M., Hansson Mild, K. (2005). Case-control study on cellular and cordless telephones and the risk for acoustic neuroma or meningioma in patients diagnosed 2000–2003. *Neuroepidemiology* 25(3):120–128.
- Hardell, L., Carlberg, M., Mild, K. H. (2006). Case-control study of the association between the use of cellular and cordless telephones and malignant brain tumors diagnosed during 2000–2003. *Environ. Res.* 100(2):232–241.
- Hardell, L., Carlberg, M., Soderqvist, F., et al. (2007). Long-term use of cellular phones and brain tumours: increased risk associated with use for > or = 10 years. *Occup. Environ. Med.* 64(9):626–632.
- Henshel, D. S., DeWitt, J., Troutman, A. (2003). Using chicken embryos for teratology studies. *Curr. Protoc. Toxicol.* 13–14:11–19.
- Hyland, G. J. (2000). Physics and biology of mobile telephony. *Lancet* 356(9244):1833–1836.
- Iakymenko, I. L. (2001). Intensity of lipid peroxidation and concentration of free radicals in quail liver exposed to low-intensity laser irradiation to the embryo. *Ukr. Biokhim. Zh.* 73(2):87–90.
- ICNIRP (1998). Guidelines for limiting exposure to time-varying electric, magnetic and electromagnetic fields (up to 300 GHz). *Health Phys.* 74(4):494–522.
- Ingole, I. V., Ghosh, S. K. (2006). Exposure to radio frequency radiation emitted by cell phone and mortality in chick embryos (*Gallus Domesticus*). *Biomed. Res.* 17(3):205–210.
- Inouye, M., Galvin, Jr., M. J., McRee, D. I. (1982). Effects of 2.45 GHz microwave radiation on the development of Japanese quail cerebellum. *Teratology* 25(1):115–121.
- Ivanov, A. A., Grigor'ev Iu, G., Mal'tsev, V. N., et al. (2010). Autoimmune processes after long-term low-level exposure to electromagnetic fields (the results of an experiment). Part 3. The effect of the

- long-term non-thermal RF EMF exposure on complement-fixation antibodies against homogenous tissue. *Radiats. Biol. Radioecol.* 50(1):17–21.
- Kamata, H., Hirata, H. (1999). Redox regulation of cellular signalling. *Cell Signal* 11(1):1–14.
- Lai, H., Singh, N. P. (1997). Melatonin and a spin-trap compound block radiofrequency electromagnetic radiation-induced DNA strand breaks in rat brain cells. *Bioelectromagnetics* 18(6):446–454.
- Leloup, C., Magnan, C., Benani, A., et al. (2006). Mitochondrial reactive oxygen species are required for hypothalamic glucose sensing. *Diabetes* 55(7):2084–2090.
- Mattson, M. P. (2008). Hormesis and disease resistance: activation of cellular stress response pathways. *Hum. Exp. Toxicol.* 27(2):155–162.
- Merry, T. L., Steinberg, G. R., Lynch, G. S., McConell, G. K. (2010). Skeletal muscle glucose uptake during contraction is regulated by nitric oxide and ROS independently of AMPK. *Amer. J. Physiol. Endocrinol. Metab.* 298(3):E577–E585.
- Nemoto, S., Takeda, K., Yu, Z. X., et al. (2000). Role for mitochondrial oxidants as regulators of cellular metabolism. *Mol. Cell. Biol.* 20(19):7311–7318.
- Ozguner, F., Altinbas, A., Ozaydin, M., et al. (2005). Mobile phone-induced myocardial oxidative stress: protection by a novel antioxidant agent caffeic acid phenethyl ester. *Toxicol. Ind. Health* 21(9):223–230.
- Ozgur, E., Guler, G., Seyhan, N. (2010). Mobile phone radiation-induced free radical damage in the liver is inhibited by the antioxidants N-acetyl cysteine and epigallocatechin-gallate. *Int. J. Radiat. Biol.* 86(11):935–945.
- Repacholi, M. H., Basten, A., Gebiski, V., et al. (1997). Lymphomas in E mu-Pim1 transgenic mice exposed to pulsed 900 MHz electromagnetic fields. *Radiat. Res.* 147(5):631–640.
- Ristow, M., Zarse, K. (2010). How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis). *Exp. Gerontol.* 45(6):410–418.
- Sadetzki, S., Chetrit, A., Jarus-Hakak, A., et al. (2008). Cellular phone use and risk of benign and malignant parotid gland tumors—a nationwide case-control study. *Amer. J. Epidemiol.* 167(4):457–467.
- Saito, K., Suzuki, K., Motoyoshi, S. (1991). Lethal and teratogenic effects of long-term low-intensity radio frequency radiation at 428 MHz on developing chick embryo. *Teratology* 43(6):609–614.
- Santini, R., Santini, P., Danze, J. M., et al. (2002). Study of the health of people living in the vicinity of mobile phone base stations: 1. Influences of distance and sex. *Pathol. Biol.* 50:369–373.
- Sarkar, S., Ali, S., Behari, J. (1994). Effect of low power microwave on the mouse genome: a direct DNA analysis. *Mutat. Res.* 320(1–2):141–147.
- Stebbing, A. R. (1982). Hormesis—the stimulation of growth by low levels of inhibitors. *Sci. Total Environ.* 22(3):213–234.
- Szmigielski, S., Szudziński, A., Pietraszek, A., et al. (1982). Accelerated development of spontaneous and benzopyrene-induced skin cancer in mice exposed to 2450-MHz microwave radiation. *Bioelectromagnetics* 3(2):179–191.
- Tkalec, M., Malaric, K., Pevalek-Kozlina, B. (2007). Exposure to radiofrequency radiation induces oxidative stress in duckweed Lemna minor L. *Sci. Total Environ.* 388(1–3):78–89.
- Tsybulin, O., Yakymenko, I. (2009). Effect of non-thermal optic electromagnetic radiation on embryo development of quails. *Photobiol. Photomed.* 3:75–85.
- Valko, M., Leibfritz, D., Moncol, J., et al. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.* 39(1):44–84.
- Vinogradov, G. I., Dumansky, Y. D. (1974). Change in the antigenic properties of tissues and autoallergic processes in case of the action of ultra-high frequency energy. *Bull. Exp. Biol. Med.* 78(8):76–79.
- Volkow, N. D., Tomasi, D., Wang, G. J., et al. (2011). Effects of cell phone radiofrequency signal exposure on brain glucose metabolism. *JAMA* 305(8):808–813.
- Weisbrot, D., Lin, H., Ye, L., et al. (2003). Effects of mobile phone radiation on reproduction and development in *Drosophila melanogaster*. *J. Cell. Biochem.* 89(1):48–55.
- Wolf, R., Wolf, D. (2007). Increased incidence of cancer near a cell-phone transmitted station. In: Columbus, F. (ed.) *Trends in Cancer Prevention* (pp. 1–8). New York: Nova Science Publishers, Inc.
- Yakymenko, I., Besulin, V., Testik, A. (2002). The effects of low intensity red laser irradiation on hatching eggs in chicken and quail. *Int. J. Poul. Sci.* 1(1–3):06–08.
- Yakymenko, I., Henshel, D., Sidorik, E., et al. (2011). Effect of mobile phone electromagnetic radiation on somitogenesis of birds. *Rep. NAS Ukraine*(1):146–152.
- Yakymenko, I., Sidorik, E. (2010). Risks of carcinogenesis from electromagnetic radiation of mobile telephony devices. *Exp. Oncol.* 32(2):54–60.
- Yang, L., Wang, M., Hao, D., Zeng, Y. (2010). Cellular canceration induced by mobile phone radiation. *Bioinformatics and Biomedical Engineering (iCBBE)*, 2010 4th International Conference, June 18–20, pp. 1–4. Chengdu, China; Date of Current Version: 23 July 2010.
- Zareen, N., Khan, M. Y., Ali Minhas, L. (2009). Derangement of chick embryo retinal differentiation caused by radiofrequency electromagnetic fields. *Congenit. Anom. (Kyoto)* 49(1):15–19.