

GSM 900 MHz cellular phone radiation can either stimulate or depress early embryogenesis in Japanese quails depending on the duration of exposure

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Abstract

Purpose: Our study was designed to assess the effects of low intensity radiation of a GSM (Global System for Mobile communication) 900 MHz cellular phone on early embryogenesis in dependence on the duration of exposure.

Materials and methods: Embryos of Japanese Quails were exposed *in ovo* to GSM 900 MHz cellular phone radiation during initial 38 h of brooding or alternatively during 158 h (120 h before brooding plus initial 38 h of brooding) discontinuously with 48 sec ON (average power density 0.25 $\mu\text{W}/\text{cm}^2$, specific absorption rate 3 $\mu\text{W}/\text{kg}$) followed by 12 sec OFF intervals. A number of differentiated somites were assessed microscopically. Possible DNA damage evoked by irradiation was assessed by an alkaline comet assay.

Results: Exposure to radiation from a GSM 900 MHz cellular phone led to a significantly altered number of differentiated somites. In embryos irradiated during 38 h the number of differentiated somites increased ($p < 0.001$), while in embryos irradiated during 158 h this number decreased ($p < 0.05$). The lower duration of exposure led to a significant ($p < 0.001$) decrease in a level of DNA strand breaks in cells of 38-h embryos, while the higher duration of exposure resulted in a significant ($p < 0.001$) increase in DNA damage as compared to the control.

Conclusion: Effects of GSM 900 MHz cellular phone radiation on early embryogenesis can be either stimulating or deleterious depending on the duration of exposure.

Keywords: Electromagnetic field, microwave radiation, embryo, somitogenesis, DNA damage

Introduction

On 31 May 2011 the International Agency for Research on Cancer on behalf of the World Health Organization classified radiofrequency electromagnetic fields as being possible

carcinogenic to humans (Baan et al. 2011). This decision, reportedly based on an increased risk for glioma development associated with the usage of wireless phones, can serve as an indicator for the importance of research on risk assessment on modern wireless technologies. Indeed, mobile communication devices intensively spread worldwide has become the most powerful source of non-ionizing electromagnetic radiation in the human environment.

Potential risk of microwaves (MW) for human health is closely connected to contemporary approaches to define safety limits for non-ionizing electromagnetic radiation. According to the International Commission on Non-Ionizing Radiation Protection recommendations (ICNIRP 1998), safety limits were adopted solely on thermal effects of short-term exposure of biological tissues to electromagnetic fields. Nevertheless, a great amount of both epidemiological and experimental data available nowadays points to non-thermal effects of MW on biological systems. Accordingly, an increased risk of carcinogenesis was demonstrated after long-term or 'heavy' use of mobile communication devices (Eger et al. 2004, Hardell et al. 2007, Hardell and Carlberg 2009, Khurana et al. 2009, Morgan 2009). On the other hand, the Interphone study reports demonstrate a decreased risk of cancer development for 'light' users of cell phones (Cardis et al. 2010). However, the underlying mechanisms of health effects revealed still remain unclear. In this connection, experimental data on clear dependence of mutagenic effect of the low intensity MW exposure on the level of MW energy is particularly important (Phillips et al. 1998). While the mutagenic effect of non-ionizing radiation is viewed as one of the most important experimental evidence of its potential carcinogenicity (Behari and Paulraj 2007), these data deserve special attention. Importantly, a few relevant studies have already demonstrated dependence of the biological effects of MW on the intensity of

radiation (in non-thermal range). In particular, the oxidative and mutagenic effects of MW were convincingly shown on a model of human spermatozoa (De Iuliis et al. 2009). In addition, the intensity-dependent biological effects of GSM MW were revealed on a model of *Drosophila melanogaster* (Panagopoulos et al. 2010). Likewise, we have earlier demonstrated that exposure of quail embryos *in ovo* to MW of GSM 850 MHz with intensity 5–15 $\mu\text{W}/\text{cm}^2$ led to distinct malformations of somites in 40-h embryos (Yakymenko et al. 2011c).

On the other hand, we recently learned that the 38-h exposure to extremely low intensity MW (0.2 $\mu\text{W}/\text{cm}^2$) can result in the facilitating effects on somitogenesis of quail embryos (Tsybulin et al. 2012). Therefore, in the present study we investigated whether prolongation of the exposure to MW of the same extremely low intensity can lead to any opposite effect on somitogenesis. Additionally, possible mutagenic effects of different durations of the low intensity MW exposure of embryonic cells were assessed.

It is important to note that in this study a commercial model of a cellular phone of the GSM standard was used as a source of radiation. To that, GSM is currently a prevailing standard for mobile communication, which covers about 80% of all services around the world. The frequencies of electromagnetic waves used in GSM are about 850, 900, 1800 or 1900 MHz, which all belong to a microwave range. Using them, information is transferred via modulation of electromagnetic wave frequency. In the GSM standard TDMA (Time Division Multiple Access) principle is realized, which means a part time access to the logical channel with frequency of channels' rotation at about 217 Hz (Hyland 2000).

Importantly, commercial models of cellular phones are widely used nowadays in risk assessment studies (see for example Ilhan et al. 2004, Meral et al. 2007, Agarwal et al. 2009, Volkow et al. 2011). Indeed, one of the most intriguing questions is whether any detectable biological/health effects can be caused by the radiation from typical commercial wireless devices under the existing safety limits. Our data demonstrate such effects and consequently give an affirmative answer to this question. In addition, our results suggest that the various durations of low intensity MW irradiation evoke robust biological effects, which vary in their directions.

Materials and methods

Incubation of embryos

Embryos of Japanese quail were used in this study. Fresh fertilized Japanese quail eggs were purchased from Bila Tserkva Poultry Farm (Bila Tserkva, Ukraine). For each experiment two groups of eggs qualified to the incubation standards were formed ($n = 10\text{--}12$). Brooding of embryos was initiated by placing the eggs into a laboratory incubator ILU-F-0.3 (Pyatigorskseilmach, Pyatigorsk, Russia) under conditions described as optimal for quail embryos: Temperature of incubation $38.3 \pm 0.2^\circ\text{C}$, relative humidity 60% (Yakymenko et al. 2002). Horizontal trays for incubation and manual rotation of eggs three times per day were used. Metal walls of the incubator were replaced by plastic

covers to avoid shielding or reflection of MW. The exposed and control groups of embryos were incubated simultaneously in the same incubator chamber, separated from each other by 10 cm, while the control group was shielded by six layers of an aluminum foil (each foil layer was 0.03 mm thick, $20 \times 30 \text{ cm}^2$).

Irradiation of embryos

We intended to design our strategy in order to compare biological effects of MW irradiation in a relation to the duration of exposure, and hence to a received dosage of irradiation. We reasoned that various exposures applied during the period of actual brooding of the eggs would result in embryos at different developmental stages which would make the comparisons difficult if possible. Therefore, to prolong the period of exposure we irradiated embryos *in ovo* at a 'resting' stage in addition to irradiation at the actual brooding stage. We based this strategy on our previous studies which demonstrated high sensitivity of bird embryos to non-ionizing radiation exposure applied both before and during the initial period of incubation (Yakymenko et al. 2002, Tsybulin and Yakymenko 2009).

A commercial cell phone (Nokia 3120, Bochum, Germany) assigned to a local mobile connection provider (Kyivstar, Kyiv, Ukraine) was used as a source of GSM 900 MHz radiation. Activation of the muted and silenced cell phone was performed by auto-redial computer program AutoRingUp (Special Software, Tomsk, Russia). The program ensured redial of the cell phone number after disconnection of a previous call. Each dial cycle consisted of a connection attempt (cell phone ON) which lasted for about 48 sec followed by 12 sec OFF interval. During each connection trial (without 'answering' a call) the phone emitted MW radiation at 890–915 MHz carrier frequency. During the call a GSM signal is non-modulated by any voice signal while maintaining a pulse modulation which is equivalent to an amplitude modulation simultaneously by several frequencies (217 Hz; 434 Hz; 651 Hz, etc). However, there is no detectable amplitude modulation at the frequency of 217 Hz (Stewart 2000). The phone rested on a plastic stand 3 cm over the surface of eggs. All experiments were carried out at the same place of the laboratory that ensured a stable position of a cell phone relatively to a nearest base transceiver station. As a result, relatively stable level of radiation from the cell phone throughout the experimental series was obtained (Figure 1).

Radiofrequency (RF) Field Strength Meter (Alphalab Inc, Salt Lake City, UT, USA) was used for measurement of power density of MW radiation. The intensity of MW radiation varied significantly during the time of the calls while still maintaining a reproducible profile (Figure 1). An average power density of MW radiation on the surface of incubation eggs during the calls was $0.25 \pm 0.008 \mu\text{W}/\text{cm}^2$. The level of background RF radiation in the laboratory was $0.001 \mu\text{W}/\text{cm}^2$ (in range of 100–3000 MHz). The aluminum foil shield used in our experiments ensured near-the-background level of RF radiation ($0.002 \mu\text{W}/\text{cm}^2$) for the control quail embryos while cell phone was under the operation conditions in a zone of exposed embryos.

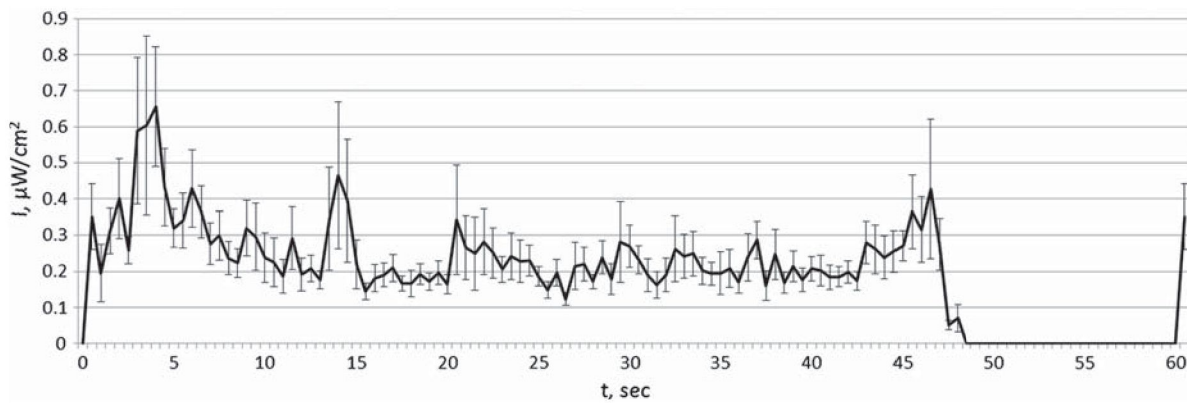


Figure 1. Plot of the intensity of GSM 900 MHz microwaves emitted from a cellular phone Nokia 3120 during a calling cycle at 3 cm from the back of the phone. Intensities were recorded at various hours during the day inside the incubator, the place at the laboratory space was not changed throughout the experiment series; $n = 9$; the mean \pm the standard error of the mean (SEM).

The level of specific absorption rate (SAR) in quail embryo *in ovo* for applied MW radiation was assessed as $(\sigma/\rho)E^2$ according to (Chou et al. 1996, Cespedes and Ueno 2009), where $\sigma \approx 70$ S/m is conductivity of embryo/hatching egg structures, $\rho \approx 1040$ kg/m³ is an average density of quail embryo/hatching egg structures and $E \approx 0.02$ V/m is the electric field inside the hatching egg. The last number was calculated through the equation $S = E^2/377$ (see, for example, ICNIRP 1998), where S is a power density of MW in W/m², and E is electric field inside hatching egg, keeping in mind that average power density on the egg surface in our experiments was 0.0025 W/m² (0.25 μ W/cm²) and permittivity of hatching egg structures is about 50 for MW range (Eroshenko et al. 2005). The calculated value of SAR was about 3 μ W/kg, which is far below the ICNIRP limit in 2 W/kg (ICNIRP 1998).

Embryos of the Exposed I experimental groups (lower dosage) were irradiated by the phone *in ovo* during the first 38 h of brooding in an incubator as shown in Figure 2. Embryos of the Exposed II groups (higher dosage) were first irradiated on a laboratory bench at room temperature during 5 days (120 h), after which eggs were transferred to the incubator where irradiation continued for 38 h similarly as in Exposed I groups (158 h of irradiation in total). It is of note here that in fresh fertilized quail eggs embryos stay in a stage of a gastrula which consists of a few hundred cells and

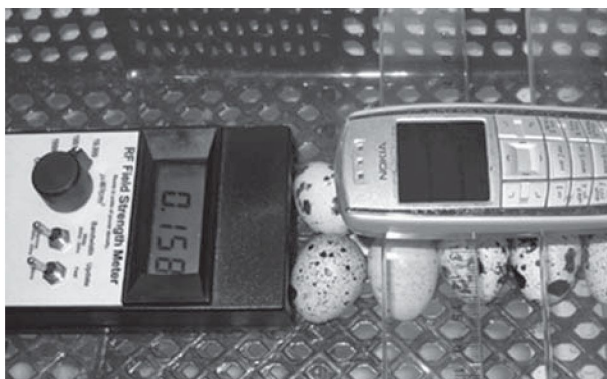


Figure 2. Set up for irradiation of quail embryos *in ovo* (exposed group) to MW emitted by a commercial GSM 900 MHz cell phone (magnification $\times 0.5$).

which are to continue their development if placed under proper brooding conditions. The temperature on the surface of hatching eggs of both the control and exposed groups was controlled by thermometers with a 0.1°C precision.

Analysis of the intensity of somitogenesis

A number of differentiated somites in a bird embryo are well known as one of the most objective integral index of early embryonic development (Hamburger and Hamilton 1992). Analysis was carried out as described (Yakymenko et al. 2011c). Briefly, after 38 h of brooding embryonic development was stopped by cooling the eggs in cold water (10°C). Embryos were taken off the surface of yolk using filter paper rings after cracking the egg shells and removal of the whites. Embryos were then washed carefully in a cold phosphate-buffered saline (PBS) (Alsi, Kyiv, Ukraine). A calculation of the numbers of differentiated somites and visual analysis of development abnormalities were carried out under a light microscope Biolam 70 (Leningrad optical-mechanical corporation, Petersburg, Russia). All unfertilized eggs were excluded from the following statistical analysis.

Analysis of DNA single- and double-strand breaks in embryo cells

Analysis of DNA single- and double-strand breaks in embryo cells was performed using an alkaline Comet assay as described (Singh et al. 1988, Hartmann et al. 2003) with slight modifications. Briefly, embryos, taken and washed as above, were detached from paper rings and cells were dissociated by careful trituration of a whole embryo in PBS to achieve about 5×10^6 of cells per ml. The frosted microscope slides were first covered with a layer of an agarose gel (Sigma-Aldrich, Munich, Germany). Then, $1-2 \times 10^5$ of cells were embedded into 75 μ l of 1% low melting point agarose (Sigma-Aldrich) at 37°C and the gel was cast over the first agarose layer on ice for 10 min. Slides were immersed into a lysis solution (2.5 M NaCl, 100 mM ethylenediamine tetra-acetic acid (EDTA), 10 mM Tris, 10% dimethylsulphoxide (DMSO), 1% Triton X-100, pH 10, all reagents Sigma-Aldrich) and kept for an hour at 4°C. After cell lysis, the slides were placed in a horizontal gel electrophoresis unit filled with alkaline electrophoresis buffer

(300 mM NaOH and 1 mM EDTA, pH 13). After 30 min of alkali treatment for unwinding the DNA, electrophoresis was performed for 20 min at 0.8 V/cm. Slides were rinsed consecutively with neutralization buffer (0.4 M Tris Cl, pH 7.5, Sigma-Aldrich) and distilled water, and stained with SYBR Green I (Sigma-Aldrich). All procedures described were conducted under dimmed light. The slides were evaluated under a fluorescence microscope (Carl Zeiss Fluoval, Jena, Germany) coupled to an image analysis system Digital Camera for Microscope DCM 500 (Hangzhou Huaxin IC Technology Inc, Hangzhou, Zhejiang, China). Analysis of the images was performed using CometScore software (TriTek Corp, Sumerduck, VA, USA). At least 50 cells were analyzed for each slide/embryo. DNA damages were assessed by calculation of tail length (μm), percentage of DNA in a tail, and a tail moment (product of the tail length to tail DNA content).

Statistical analysis

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

Results

Effects of GSM 900 MHz cell phone radiation on somitogenesis depend on the duration of exposure

In the Exposed I groups, the MW exposure of quail embryos *in ovo* by a commercial GSM 900 MHz cell phone to average power density $0.25 \mu\text{W}/\text{cm}^2$ during 38 h with 48 sec ON, 12 sec OFF intervals increased the number of differentiated pairs of somites by 14.4% ($p < 0.001$) as compared with the unexposed controls (Table I, Figure 3).

On the other hand, in the Exposed II groups an extension of the MW exposure to 158 h in total by additional irradiation

Table I. GSM 900 MHz microwaves emitted from the commercial cell phone affect somitogenesis of quail embryos. Embryos were irradiated *in ovo* either during initial 38 h of brooding (Exposed I) or during 5 days before the brooding plus initial 38 h of brooding (Exposed II). Numbers of pairs of differentiated somites were counted microscopically.

Groups	Exposure		Exposure dose, mJ/cm^2	The number of differentiated somite pairs
	Total time, hours	Power density, $\mu\text{W}/\text{cm}^2$		
Exposed I [†]	38	0.25	27.36	$13.31 \pm 0.26^{***}$
Control I [†]	-	-	-	11.63 ± 0.25
Exposed II ^{††}	158	0.25	113.76	$9.78 \pm 0.69^*$
Control II ^{††}	-	-	-	11.45 ± 0.27

[†] $n = 26$, combined data of three experiments; the mean \pm SEM; ^{††} $n = 32$, combined data of four experiments; the mean \pm SEM; * $p < 0.05$; *** $p < 0.001$ as compared with the matched control.

of embryos for 120 h at room temperature before actual 38 h brooding decreased the number of pairs of differentiated somites by 14.6% ($p < 0.05$) as compared with the matched controls (Table I, Figure 3).

Importantly, the equal number of differentiated somites in the control groups in both series of experiments was observed. Therefore, five-day storage of hatching eggs before the actual brooding affected neither viability nor development potential of embryos, which corresponds to the incubation standards (Petek et al. 2003).

Microscopic analysis of embryos did not reveal any developmental abnormalities neither in the control, nor in the exposed groups. Measurement of temperature at the surface of hatching eggs did not show any differences between the exposed and the control groups (not shown).

Taken together, these data show that radiation emitted by a commercial GSM 900 MHz cell phone can either increase or decrease the number of differentiated somites in developing embryos depending on the duration of the exposure.

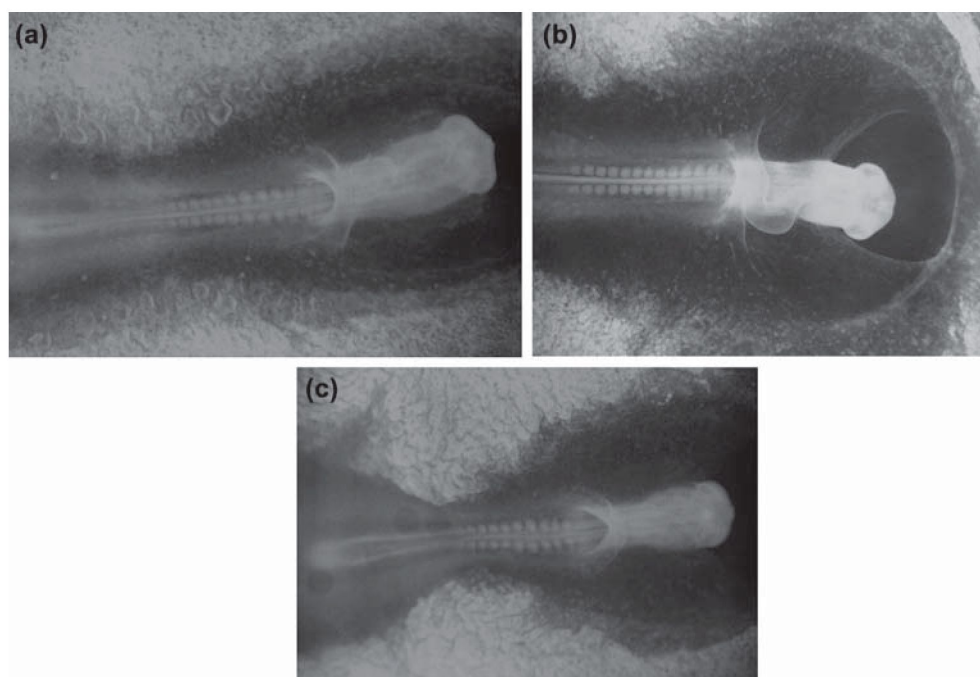


Figure 3. Microscopic pictures of 38 h quail embryos, ventral view (magnification $\times 24$). (a) Control; (b) Exposed I (exposure during initial 38 h of brooding); (c) Exposed II (exposure during 5 days before the brooding plus initial 38 h of brooding).

Table II. GSM 900 MHz microwaves emitted from the commercial cell phone affect DNA integrity in cells of quail embryos. Embryos were irradiated *in ovo* either during initial 38 h of brooding (Exposed I) or during 5 days before the brooding plus initial 38 h of brooding (Exposed II). Alkaline comet assay was used for an assessment of DNA single- and double-strand breaks in dissociated embryonic cells.

Indexes	Exposed I†	Control I†	Exposed II††	Control II††
Exposure dose, mJ/cm ²	27.36	-	113.76	-
Tail length (μm)	5.38 ± 0.35***	11.69 ± 0.87	21.20 ± 0.60***	12.13 ± 0.57
DNA in tail, %	19.16 ± 0.88***	25.38 ± 1.35	29.24 ± 0.68***	22.32 ± 0.76
Tail moment	1.90 ± 0.27***	5.55 ± 0.69	8.55 ± 0.39***	4.52 ± 0.38

†50 cells from each of 5 embryos were analyzed; the mean ± SEM; ††100 cells from each of 5 embryos were analyzed; the mean ± SEM; *** $p < 0.001$ as compared with the matched control.

Effects of GSM 900 MHz cell phone radiation on integrity of DNA in cells of quail embryos depend on the duration of exposure

In the Exposed I group, MW exposure of embryos *in ovo* by a commercial GSM 900 MHz cell phone significantly decreased ($p < 0.001$) single- and double-strand DNA breaks analyzed by an alkaline comet assay (Table II, Figure 4). Indeed, both the tail length and tail moment were more than twice lower as compared with the relative control indices, while percentage of DNA in tails was 24.5% lower.

On the other hand, in the Exposed II group, prolongation of exposure for 5 days before actual 38 h brooding resulted in a significant ($p < 0.001$) increase of DNA damage in embryonic cells (Table II, Figure 4). Thus, indices of both tail length and tail moment in cells of the Exposed II group embryos were almost twice higher, while percentage of DNA in tails was 31.2% higher as compared with the corresponding control groups.

To correctly assess any possible effects of the five-day storage of hatching eggs before actual brooding we compared

the level of DNA strand breaks in the Control I to Control II embryos. The observed values were slightly different and reached statistical significance only for percentage of DNA in tails ($p < 0.05$). However, this difference was substantially lower than between the corresponding exposed and the control groups. Noteworthy, we observed high level of DNA damages in control quail embryo cells as compared to others biological models (Diem et al. 2005). This probably reflects biological commitment of cells in the early embryo to intensive proliferation which obviously might affect the state of their DNA.

Taken together, this suggests that the low intensity radiation emitted by a commercial GSM 900 MHz cell phone can result in either decrease of DNA strand breaks; or otherwise induce substantial DNA damage in cells of the developing bird embryo depending on the duration of the exposure.

Discussion

Worldwide expansion of mobile communication technology during the last decades dramatically increased the

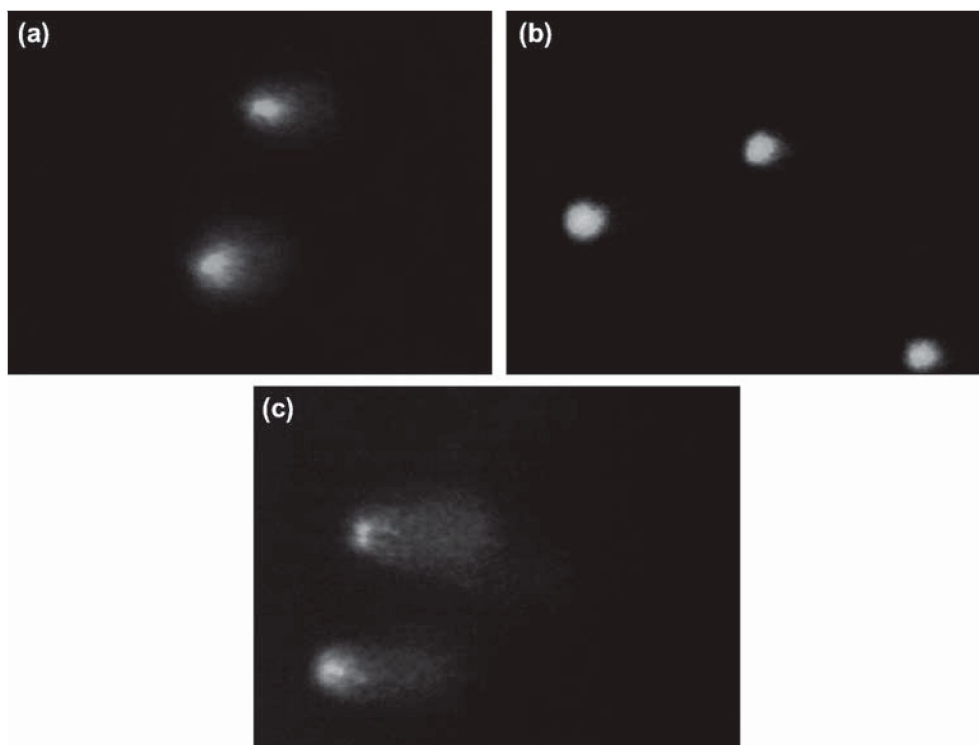


Figure 4. Microscopic pictures of alkaline comet assay on cells from 38 h quail embryos (magnification $\times 40$). (a) Control; (b) Exposed I (exposure during initial 38 h of brooding); (c) Exposed II (exposure during 5 days before the brooding plus initial 38 h of brooding).

level of chronic exposure of the human population and the environment to radiofrequency electromagnetic fields of microwave range. It is important to underline that the modern international safety limits for RF were developed based solely on thermal effects of short-term exposure of human body (ICNIRP 1998). However, numerous lines of evidences strongly suggest the non-thermal biological effects of RF/MW (reviewed in, e.g., Belyaev 2010, Yakymenko et al. 2011a). Therefore, it seems to be reasonably substantiated that national safety limits in many countries are currently much stricter than the ICNIRP recommendations. For example, RF power density for general public is restricted to 2.5 $\mu\text{W}/\text{cm}^2$ in Ukraine, 4 $\mu\text{W}/\text{cm}^2$ in Switzerland and to 10 $\mu\text{W}/\text{cm}^2$ in Russia, China and Italy (Ministry of Health of Ukraine 1996, Hardell and Sage 2008) as compared to 450–1000 $\mu\text{W}/\text{cm}^2$ recommended by ICNIRP (ICNIRP 1998). However, even those tight national safety limits often do not regulate the duration of RF exposure, operating only with the intensity; and neither apply safety approaches based on the dosage of energy. Nevertheless, these factors can be crucial for mobile communication users, especially for children and young adults as nowadays they get exposed to MW continuously in their life, sometimes as much as for a few hours of phone conversations a day (Yakymenko et al. 2011b). A great importance of the duration of exposure in risk assessment of MW effects was convincingly demonstrated in a recent report (Schwarz et al. 2008). On the other hand, a distinctive stimulating and even healing biological effects of MW in optimized doses have been described as well (Belyaev 2010). This indicates a complexity in the intimate mechanisms of interaction of the low intensity MW with the biological systems.

Recently we demonstrated a mild facilitating effect of the low intensity GSM 900 MHz exposure of quail embryos on somitogenesis (Tsybulin et al. 2012). This effect was achieved with MW at 0.2 $\mu\text{W}/\text{cm}^2$ power density. Importantly, this is three orders of magnitude below the actual ICNIRP safety limits. Therefore, the aim of the present study was to elucidate whether prolongation of exposure with the same low intensity can result in aggravation of its effects on early embryos. Indeed, our results show that extended exposure to cell phone radiation can inhibit the early embryonic development and induce DNA single- and double-strand breaks in embryonic cells. Previously a great importance of the duration of MW exposure in genotoxic effects was convincingly demonstrated in a study of Schwarz et al. (2008) on an *in vitro* model of human fibroblasts.

Importantly, the extremely low intensity of radiation and thermal control during the experiments exclude thermal mechanism of effects revealed in our study. Moreover, a conventional thermal effect within a biologically acceptable temperature limits leads to an acceleration of bird embryo development but not to depression of it.

Mechanisms of biological effects of the low intensity MW are currently intensively studied. In particular, many experimental data indicate an involvement of reactive oxygen species (ROS) in the induction of the detrimental effects of MW (Ozguner et al. 2005, 2010, Friedman et al. 2007, Agarwal et al. 2009, De Iuliis et al. 2009, Kesari et al. 2011,

Avci et al. 2012). Reportedly, both mitochondrial and NADH oxidase pathways of ROS generation can be activated under the certain regimens of MW exposure (Friedman et al. 2007, De Iuliis et al. 2009). It has been experimentally proven that under MW exposure an excessive ROS generation can result in oxidative damage of DNA (De Iuliis et al. 2009) and other adverse effects up to the cell death (Salford et al. 2003, Bas et al. 2009). Similarly, such intrinsic metabolic changes could be behind observed retardation of early embryonic development revealed by inhibited somitogenesis. In addition, earlier we showed a significant activation of peroxidation processes in tissues of quail embryos after 14-day exposure at 0.2 $\mu\text{W}/\text{cm}^2$ power density (Tsybulin et al. 2012).

On the other hand, an intriguing question remains about the mechanisms of the facilitating effects of the low intensity MW exposure confirmed in the present study. Previously we suggested that a 'classical' hormetic effect (Calabrese 2008) is behind the observed stimulatory activity of low intensity MW (Tsybulin et al. 2012). Indeed, certain potentially adverse metabolic changes at under-threshold levels can activate various defense/repair mechanisms, including antioxidant and detoxification systems, which might result in facilitating effects. Apparently, this concept can also be applied for an explanation of the acceleration of early embryogenesis and decrease in DNA damages observed in the present study. Importantly, either increase or decrease of DNA damage levels depending on MW power density was earlier revealed in lymphoblastoid cells (Phillips et al. 1998).

We designed this study to reveal possible biological effects of radiation emitted by a commercial cellular phone used nowadays routinely by the general public. It is noteworthy that a commercial model of a cell phone produces a rather complex non-uniform signal in the microwave range. Moreover, a close vicinity of a cell phone to an exposed object like in our experiments results in an additional exposure to extremely low frequency (ELF) magnetic field due to electric currents in a battery circuit of a cell phone. Obviously, the use of a commercial model of a cell phone provides unique opportunities for studies on risk assessment. On the other hand, it makes it difficult to standardize the exposure applied. Thus, although we carefully assessed the level of MW signals during the exposure, the evaluation of the ELF magnetic field was left beyond the scopes of the present study. Reportedly, ELF magnetic field nearby a cell phone during its operation can reach from a few μT up to a few tens μT of magnitude (Stewart 2000, Einat and Yahalom 2011). The latter is comparable to the international safety limits for ELF magnetic field, which, for example, for frequency 50 Hz is 100 μT (ICNIRP 1998). Consequently, certain modulating effects of magnetic fields could have added to the biological phenomena revealed in our experiments. The issue of the biological influence of magnetic fields is currently under consideration for a follow up study.

In addition, one of the intriguing questions for a follow-up study is to investigate long-term effects of early embryo exposure. Importantly, as the bird embryo is generally accepted as a sensitive and useful model for risk assessment (Henshel et al. 2003), our data can be considered with

certain restrictions for assessing interaction of MW with the human tissues. It is important to note that the distance from a cell phone to the irradiated embryos used in our experiments was close to that from a cell phone to an inner ear or closest parts of brain of a person during a cell phone conversation without a hands-free device, or, say, to genitalia when a user keeps a cell phone in a pocket. Moreover, we can conclude that human fetuses can also be potentially affected by sharply increased public MW over-exposure. Importantly, some epidemiological studies have demonstrated a statistically significant increase in the risks of behavioral problems in children after prenatal MW exposure (Divan et al. 2008). Likewise, recent experimental study showed altered neurodevelopment and behavior in mice after cell phone MW exposure of fetuses (Aldad et al. 2012).

Taken together, our data demonstrate that changing the duration of exposure to GSM 900 MHz cellular phone radiation in a model of early embryogenesis can result in various detectable biological phenomena with the possible adverse effects.

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Declaration of interest

The authors report no declaration of interest. The authors alone are responsible for the content and writing of the paper.

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