

## OXIDATIVE AND MUTAGENIC EFFECTS OF LOW INTENSITY MICROWAVE RADIATION ON QUAIL EMBRYOS

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*Intensive implementation of wireless communication systems raised the question of the possible adverse effects of anthropogenic electromagnetic radiation. This study aims to assess the biological effects of low intensity microwaves (MW) radiation from smartphone Huawei Y51 commercial model used alone or in combination with attached Waveex chip that balances low frequency electromagnetic field but does not affect microwave signal. The biological model of developing quail embryos in ovo was used in the study. The phone as a source of low intensity 1800 MHz (0.32  $\mu\text{W}/\text{cm}^2$ ) microwaves radiation was placed at 3 cm over the surface of hatching eggs and discontinuously activated with a computer program (48 s - on, 12 s - off). It was demonstrated that the exposure of quail embryos to radiation resulted in a statistically significant increase in the content of superoxide, nitrogen oxide and TBA products, DNA integrity damage in embryo cells and increased embryo mortality. Application of Waveex chip during the exposure resulted in a partial normalization of oxidative status and DNA integrity in embryonic cells indicating a negative impact not only of MW radiation, but of low-frequency electromagnetic fields from mobile devices as well.*

**Key words:** microwave radiation, mobile devices, quail embryos, reactive oxygen species, DNA, mutagenic effects.

The International Agency for Research on Cancer/the World Health Organization (WHO) classified radiofrequency radiation (RFR) in the frequency range 30 kHz-300 GHz as a possibly human carcinogen (group 2B) [1]. During recent years a large number of research on a link between various types of tumors and low intensity microwave/radiofrequency (MW/RF) radiation have been published. Reports pointed to an increased risks in brain tumors, acoustic neuroma, tumors of parotid glands, seminomas, melanomas and lymphomas among long-term users of cellular phones [2]. Other studies indicate that long-term RFR exposure in humans can cause various non-cancer disorders, e.g., headache, fatigue, depression, tinnitus, skin irritation, hormonal disorders and other conditions [3].

In addition, convincing studies on hazardous effects of RFR in human germ cells have been published [4]. Also, some research demonstrate a variety of adverse effects of low intensity RFR from wireless communication systems on wildlife and ecosystems [5, 6].

Up to date more than hundred papers have been published on mutagenic effects of RFR and most of them revealed significant effects (see, for example, [7]). The analysis of modern data on biological effects of low intensity RFR leads to a firm conclusion that this physical agent is a powerful oxidative stressor for living cells [8]. The oxidative potential of RFR can be mediated via changes in activities of key ROS-generating systems, including mitochondria and non-phagocytic NADH oxidases [9]. Likewise,

a marker of oxidative damage of DNA, 8-hydroxy-2'-deoxyguanosine (8-OH-dG) was detected in some studies on risk assessment of low intensity RFR [10].

Earlier we demonstrated a significant protective effect of low intensity monochromatic red light ( $\lambda_{\max} = 630\text{-}650$  nm) against both suppression of somitogenesis rate and oxidative stress in embryonic cells caused by MW/RFR exposure of quail embryos [11]. Here we analyze modulatory effects of Waveex chip, a magnetic field modulator, application on biological activities of low intensity MW radiation.

### Materials and Methods

Biological model of developing quail embryos *in ovo* was used for the experiments. Three group-analogues of fresh hatching eggs of Japanese Quail were formed for each experiment ( $n = 10\text{-}15$ ): 1) an unprocessed control (group C); 2) exposed to MW from a smartphone Huawei 5YII (group H); 3) exposed to MW from a smartphone with attached Waveex chip on it (group H+W). Incubation of the embryos *in ovo* was carried out in three foam plastic incubators designed for the experiments, free of metal covers. Thus, MW was neither shielded nor reflected on the incubators structures. Hatching eggs were incubated in close to optimal conditions (except of turnover three times a day instead of once per hour). All manipulations with animals were carried out according to the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986) and the First National Congress of Ukraine on Bioethics (September 2001).

*Sources of low intensity MW.* A commercial model of smartphone Huawei 5YII of the GSM 1800 MHz standard assigned to a local mobile connection provider was used as a typical source of low intensity MW from a modern wireless device. The muted and silenced smartphone was activated due to auto-redial computer program, which guaranteed a discontinuous activation of the phone as a source of MW (48 s - ON, 12 s - OFF). The phone was placed on a plastic setup 3 cm over the surface of hatching eggs of the exposed groups. MW intensities were assessed by the RF Field Strength Meter (USA).

Waveex chip (Waveex World GmbH, Austria) was used for modulation of smartphone radiation. According to the producer, Waveex technology balances out low frequency magnetic field from a cell phone and does not affect microwave signal (<http://waveexworld.net>).

In order to maximize the time of MW exposure we started irradiation of quail embryos of the exposed groups *in ovo* 5 days before the incubation (under the room temperature). Then the exposure of embryos *in ovo* was continued during 14 days of incubation. The embryos of the control group were subjected to the same procedures as the exposed ones except for the MW exposure. The exposed and control embryos were incubated in the same conditions in three separate incubators placed 2 m from each other.

The average intensity of MW on a surface of hatching eggs of exposed groups was  $0.32 \mu\text{W}/\text{cm}^2$ . A calculated specific absorption rate (SAR) value for quail embryos in our experiments was about  $3.8 \mu\text{W}/\text{kg}$ . The radiofrequency background in the laboratory during the experiments was  $0.001 \mu\text{W}/\text{cm}^2$ .

*Analysis of somitogenesis.* A number of differentiated somites in a bird embryo is well known as one of the most objective integral index of early embryonic development [12]. Analysis was carried out as described [13]. Briefly, after 38 h of brooding embryo development was stopped by cooling the eggs in cold water ( $10^\circ\text{C}$ ). Embryos were taken off the surface of yolk using filter paper rings after cracking egg shells and removal of the whites. Embryos were then washed carefully in cold phosphate-buffered saline (PBS). Calculation of numbers of differentiated somites and visual analysis of development abnormalities were carried out under a light microscope. Any unfertilized eggs revealed were excluded from the statistical analysis.

Analysis of embryo survival was done after the end of incubation and hatching as described [14]. The wastes of incubation were analyzed according to the poultry standards for Japanese quails. The eggs that failed to be hatched were opened for macroscopical observation.

Analysis of DNA single- and double-strand breaks in 38-h embryo cells was performed using an alkaline Comet assay as described [15] with slight modifications [16]. 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  $5 \times 10^6$  of cells per ml. The frosted microscope slides were first covered with a layer of an agarose gel (Sigma-Aldrich, Munich, Germany). Then,  $1\text{-}2 \times 10^5$  of cells were embedded into  $75 \mu\text{l}$  of 1% low melting point agarose (Sigma-Aldrich) at  $37^\circ\text{C}$  and the gel was cast over the first agarose layer on ice for 10 min.

Slides were immersed into a lysis solution 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. 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 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 (China). Analysis of the images was performed using CometScore software (TriTek Corp, USA). At least 50 cells were analyzed for each slide/embryo. DNA damages were assessed by calculation a percentage of DNA in a tail.

*Preparation of samples for oxidative stress analysis.* Homogenates of whole 38-h embryos or homogenates of particular organs (brains, livers and hearts) of 10-day embryos and one-day quail were extracted and used for the analysis. Fresh tissues were frozen/thawed, homogenized in ceramic homogenizer and dissolved 1:10 (strictly) in distilled water at 2°C. Then for each analysis proper preparations were continued.

The rate of superoxide generation in embryonic cells was assessed by electron spin resonance (ESR) through a spin-trapping technique using radiospectrometer RE-1307 (Russia) at a room temperature [17]. A specific spin trap 1-hydroxy-4-dimethylamino-2,2,6,6-tetramethyl-piperidin dihydrochloride (Novosibirsk Institute of Organic Chemistry, Russia) was used for trapping of superoxide and transforming it into the stable nitroxyl radical ( $g = 2.005$ ). The spin trap concentration in the samples was 0.5 mM. The ESR signal of nitroxyl radical was recorded in each sample triple with 2 min intervals. The rate of superoxide generation in the samples was measured through the dynamic of the nitroxyl radical signal and expressed in nmole per gram of wet tissue per min ( $\text{nmol g}^{-1}\text{min}^{-1}$ ).

The nitrogen oxide production in embryo cells was assessed by the EPR method using specific spin trap sodium diethyldithiocarbamate (Sigma-Aldrich, Germany) [18]. The ESR signal of stable iron nitrosyl complexes with  $g = 2.03$  was measured after 5 min incubation of the samples with the spin trap. The EPR signal was measured triple, every 2 min, in each sample using the radiospectrometer RE-1307

at liquid nitrogen temperature ( $T = 77 \text{ K}$ ). The rate of nitrogen oxide production in embryonic cells was measured through the dynamic of ESR signal with  $g = 2.03$  and expressed in nmole per gram of wet tissue per min ( $\text{nmol g}^{-1}\text{min}^{-1}$ ).

The level of 8-oxo-dG, marker of oxidative damages of DNA in the cell, was measured by solid phase extraction from the tissues of one-day old chicks. The assessment of 8-oxo-dG concentration in the samples was made spectrophotometrically at  $\lambda = 260 \text{ nm}$  [19].

Level of lipid peroxides in the embryo tissues was assessed in reaction with thiobarbituric acid (TBA) in a presence of  $\text{Fe}^{2+}$  ions was used [20]. Briefly, 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  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  to 0.5  $\mu\text{M}$ . The reaction was carried out for 30 min in test tubes placed in boiling water and stopped in cold water. Then the test tubes were centrifuged at 3,000 rpm for 10 min. The level of TBARS was measured in supernatants by spectrophotometer Specoll 11 (Germany) at  $\lambda = 532 \text{ nm}$ .

Superoxide dismutase (SOD) activity was assessed using the assay based on a competition of SOD and nitro blue tetrazolium (NBT) for superoxide [21]. Superoxide was produced in the reaction medium in a reaction of NADH with phenazine methosulfate in the presence of oxygen. A decrease of hydrazine tetrazolium level (which formed in a reaction of superoxide with NBT) due to a presence of SOD of the sample was detected spectrophotometrically at  $\lambda = 540 \text{ nm}$ .

Catalase activity assessment in the embryo tissues was made using a reaction of decomposition of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ; 0.03% solution) added into the samples. The determination of the hydrogen peroxide residual in the sample was carried out using its reaction with molibdate ammonium (4% solution) [22]. Molibdate ammonium produces with  $\text{H}_2\text{O}_2$  a color complex, which level was assessed spectrophotometrically ( $\lambda = 410 \text{ nm}$ ).

Ceruloplasmin activity in embryo cells was assessed in express test with parafenilendiamina as described in [23]. Briefly, the reaction was carried out at 60°C for 10 min and stopped by adding 25% solution of NaOH. The reaction yield was evaluated spectrophotometrically ( $\lambda = 440 \text{ nm}$ ).

*Statistical analysis.* The data were expressed as the mean  $\pm$  standard error of the mean ( $M \pm m$ ). Student's *t*-test was used for the statistical analysis,



with a significance levels  $*P < 0.05$ ,  $**P < 0.01$  and  $***P < 0.001$  as compared to the matched controls.

## Results

MW intensity from the smartphones varied significantly during the time of exposure, from 0.05 to 20  $\mu\text{W}/\text{cm}^2$ , but correlated between mobile phones that indicates on external reason, most likely the change in operation mode of the nearest base transceiver station. The average intensity of MW from the smartphones ( $0.323 \pm 0.054 \mu\text{W}/\text{cm}^2$ ) was far below the official safety limits ( $450 \mu\text{W}/\text{cm}^2$ ) in most European countries and many countries over the world. There was not detected the difference in intensity of MW from smartphones without and with Waveex chip applied.

38-hour embryos. The rate of somitogenesis was statistically significantly increased under low intensity MW radiation exposure (group H). A

number of differentiated somite pairs in this group of embryos was 13.4% higher as compared to the control group (Fig. 1). Waveex chip application normalized the effect of smartphone radiation exposure, returning the number of differentiated somites to the control level. The difference between group H and group H+W was statistically significant ( $P < 0.05$ ).

DNA double strand breaks detected in alkaline comet assay was statistically significantly (17.5%;  $P < 0.05$ ) higher in embryonic cells exposed to smartphone MW radiation as compared to the control (Fig. 2). At the same time, in cells of embryos exposed to smartphone MW radiation with Waveex chip applied, level of DNA damages was on the same level as in control embryos.

Low intensity MW radiation exposure produced significant oxidative effects in the embryo cells (group H): level of TBA-RS (indicator of lipid peroxidation) in this group was statistically signifi-

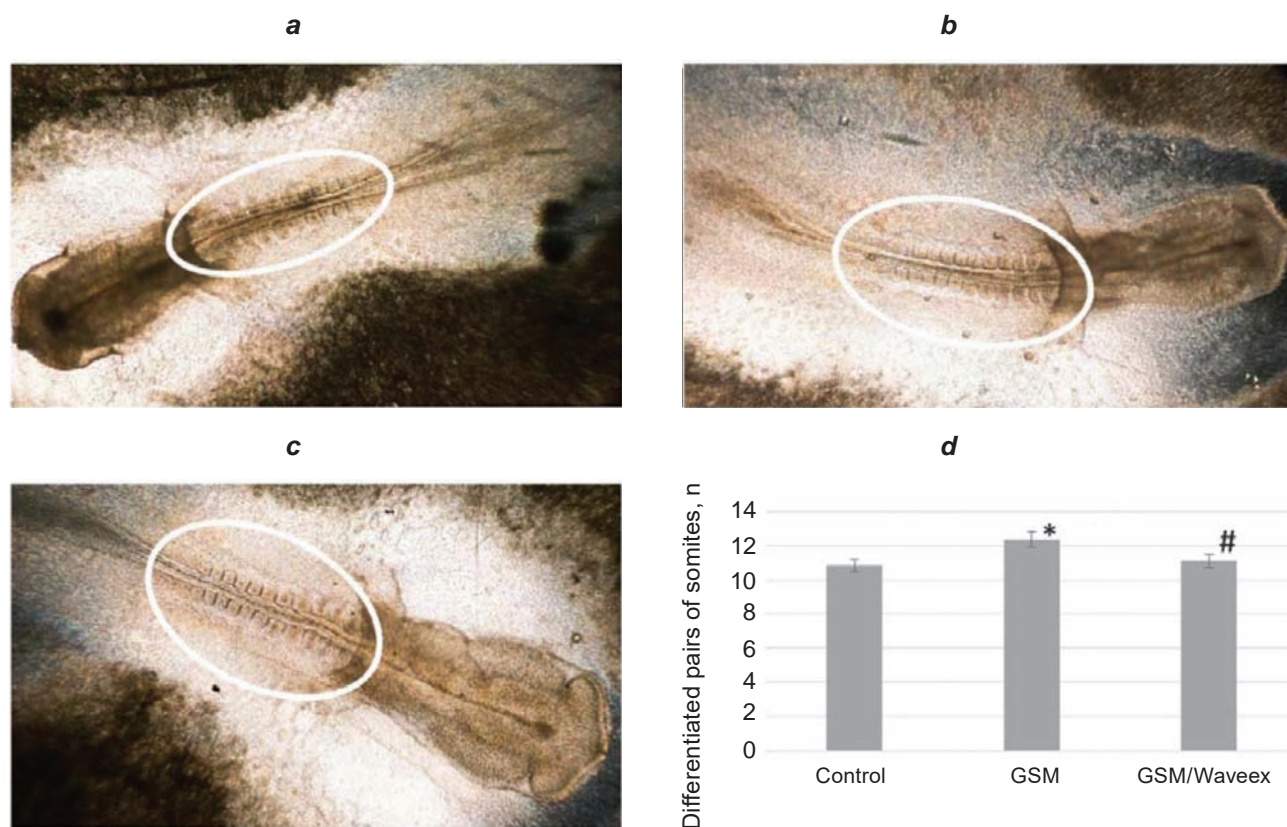


Fig. 1. Intensity of somitogenesis of 38-h quail embryos: a – microscopic picture ( $\times 24$ ) of a quail embryo of control group (11 pairs of somites); b – microscopic picture ( $\times 24$ ) of a quail embryo of GSM (13 pairs of somites); c – microscopic picture ( $\times 24$ ) of a quail embryo of GSM/Waveex (11 pairs of somites); d – differentiated pairs of somites ( $M \pm m$ ,  $n = 7$ ),  $*P < 0.05$  as compared to the control;  $\#P < 0.05$  as compared to H group. Control – an unprocessed group; GSM – exposed to MW radiation from smartphone (group H), GSM/Waveex – exposed to MW radiation from smartphone + Waveex chip (group H+W)

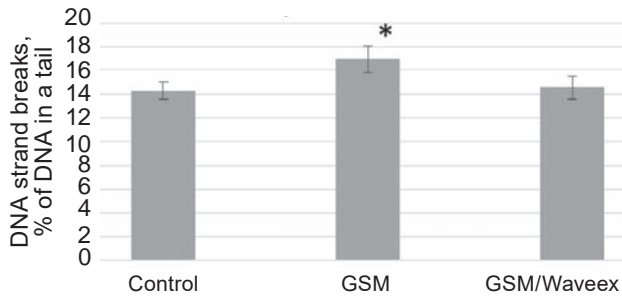


Fig. 2. DNA double strand breaks in alkaline Comet assay of living cells from 38-h quail embryos after exposure to MW radiation ( $M \pm m$ ,  $n = 5-7$ ), \* $P < 0.05$  as compared to the matched control. Control – an unprocessed group; GSM – exposed to MW radiation from smartphone (group H), GSM/Waveex – exposed to MW radiation from smartphone + Waveex chip (group H+W)

cantly higher as compared to the control, 61.1%,  $P < 0.05$  (Fig. 3). Ceruloplasmin activity in embryo cells of this group was 110% ( $P < 0.05$ ) higher than in control, and catalase activity was 60.2% higher than in control (although the last difference was not statistically significant due to significant variation of the index in the groups).

Waveex chip applied to the smartphone statistically significantly normalized oxidative status of exposed embryo cells (group H+W): TBA-RS level, activities of ceruloplasmin and catalase in cells of this group of embryos were close to control indexes. As a result, level of TBA-RS, and activity of ceru-

loplasmin in embryonic cells of H+W group were significantly ( $P < 0.05-0.001$ ) lower than in H group embryo cells (Fig. 3). Activities of SOD were not significantly changed in both exposed groups of embryos and thus were close to the control.

Ten-day embryos. Superoxide radical generation was statistically significantly, 140–210% ( $P < 0.05-0.001$ ), increased in cells of brains, hearts and livers of 10-day embryos exposed to low intensity MW radiation from the smartphone (group H) as compared to the control group of embryos (Fig. 4). The same increased level of superoxide generation was detected in group of 10-day embryos exposed to MW radiation from the mobile phone with Waveex chip applied (group H+W).

Nitrogen oxide generation also statistically significantly increased in cells of 10-day embryos after low intensity MW radiation exposure (group H), 18.7–84.7% ( $P < 0.01-0.001$ ), as compared to the control embryos (Fig. 4). Approximately the same level of nitrogen oxide generation had place in cells of 10-day embryos exposed to MW radiation from the smartphone with Waveex chip applied (group H+W). The only cells of hearts in this group of embryos had 33.4% lower generation of nitrogen oxide as compared to smartphone radiation exposed group, but difference between the groups was not statistically significant.

One-day old quail chicks. Hatchability (percent of chicks obtained from fertilized hatching eggs) was statistically significantly ( $P < 0.05$ ) decreased

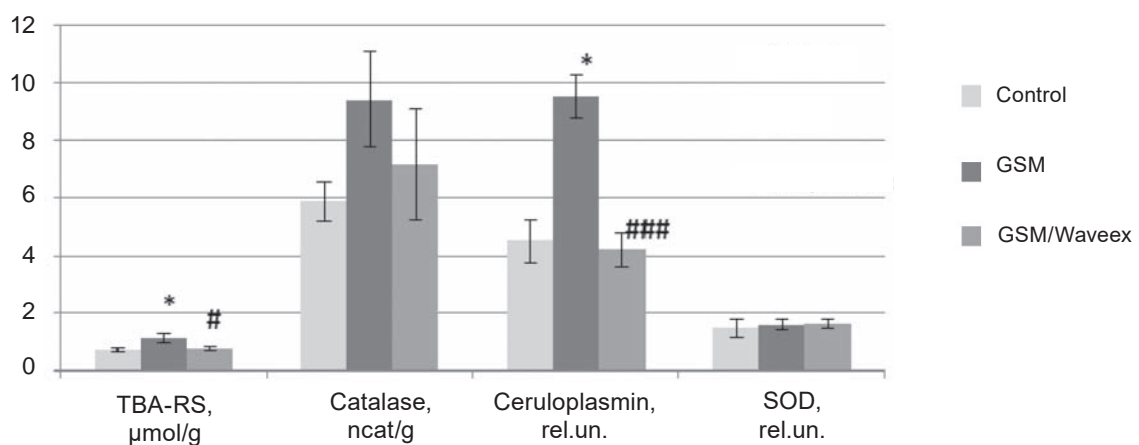


Fig. 3. The level of TBA-RS and activity of antioxidant enzymes in living cells of 38-h embryos after exposure to low intensity MW radiation ( $M \pm m$ ,  $n = 7$ ), \* $P < 0.05$  as compared to control; # $P < 0.05$  as compared to H group; ### $P < 0.001$  as compared to H group. Control – an unprocessed group; GSM – exposed to MW radiation from smartphone (group H), GSM/Waveex – exposed to MW radiation from smartphone + Waveex chip (group H+W)

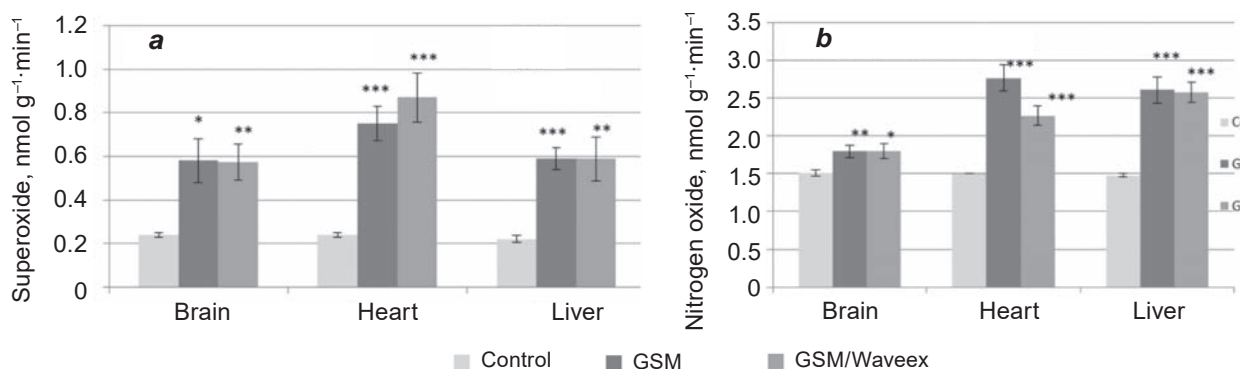


Fig. 4. The rates of superoxide (a) and nitrogen oxide (b) generation in cells of 10-day quail embryos after exposure to low intensity MW radiation ( $M \pm m$ ,  $n = 5$ ), \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  as compared to the matched controls. Control – an unprocessed group; GSM – exposed to MW radiation from smartphone (group H), GSM/Waveex – exposed to MW radiation from smartphone + Waveex chip (group H+W)

in group of eggs/embryos exposed to mobile phone MW radiation as compared to the control (20% against 57.9%). Thus, low intensity MW radiation of GSM standard from the smartphone resulted in a significant increase of embryo mortality. Hatchability in group of embryos exposed to radiation from the smartphone with Waveex chip applied was slightly higher than in H-group, (28.6% against 20%), although still much lower than in control.

Superoxide generation was significantly, 180–217% ( $P < 0.001$ ), increased in cells of brains, hearts and livers of one-day chicks from the embryos exposed to smartphone radiation, group H (Fig. 5). At the same time, chicks from the embryos exposed to smartphone radiation modulated by Waveex chip demonstrated significantly less increased level of superoxide generation, 48.3–99.6% less as compared to the H-group of embryos. Although the indexes were still statistically significantly higher than in the control, they were much closer to the control than the indexes of H-group exposed embryos.

Nitrogen oxide level in cells of brains, hearts and livers of one-day chicks from MW radiation exposed embryos of H-group was 25–87.7% higher than in control. The differences with control are statistically significant ( $P < 0.01$ – $0.001$ ) for all analyzed organs (Fig. 5). On the other hand, application of Waveex chip to mobile phone during the exposure resulted in statistically significant decrease of nitrogen oxide level (14.7–16.9%,  $P < 0.05$ – $0.01$ ) in brains and hearts of chicks as compared to the chicks from H-group exposed embryos.

The level of 8-oxo-dG, a marker of oxidative damages of DNA, statistically significantly (63.4–119.4%,  $P < 0.01$ – $0.001$ ) increased in brains,

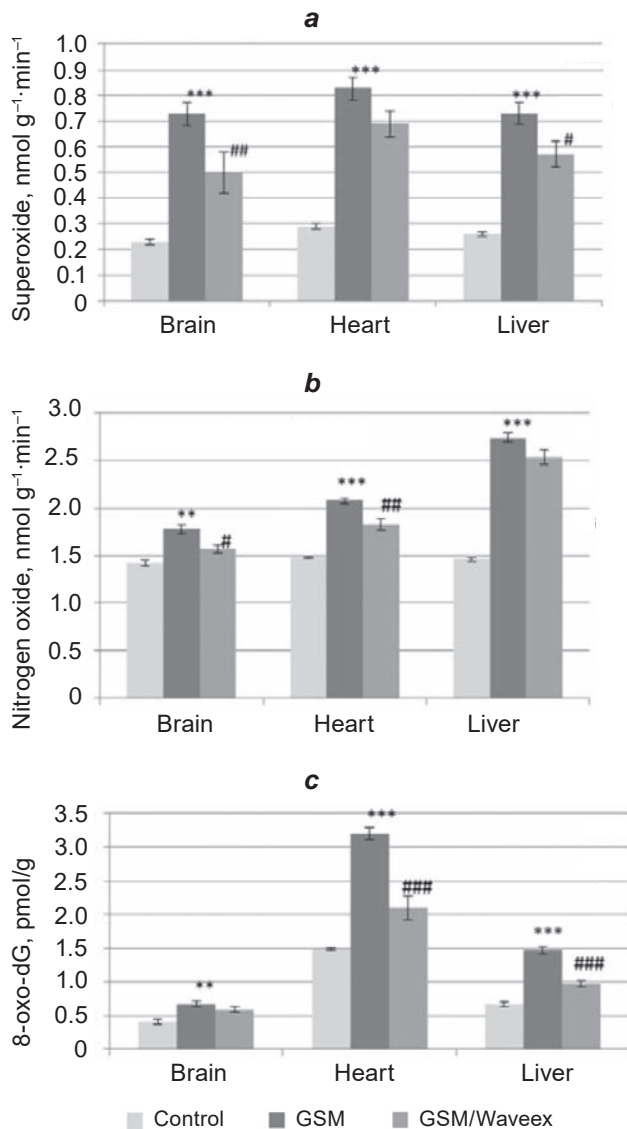
hearts and livers of one-day chicks from H-group of exposed embryos. Application of Waveex chip resulted in statistically significant decrease of 8-oxo-dG level, 73.9–74.6%, as compared to the H-group (Fig. 5).

## Discussion

Developing bird embryos can serve as a sensitive and convenient biological model for assessment of a wide range of risk factors. In our experiments, the adverse effects of low intensity MW radiation from typical commercial wireless device were persistent during the quail embryogenesis and included statistically significant changes in rate of somitogenesis, a 2-fold increased level of superoxide generation rate and up to 85% increased rate of nitrogen oxide generation in exposed embryos and hatchlings. Also, in one-day old chicks from the exposed embryos there were demonstrated statistically significant oxidative damages of DNA. Finally, low intensity MW exposure of quail embryos resulted in a significant, almost twice, increase of embryo mortality as compared to the unexposed control embryos.

It is important, that superoxide and nitrogen oxide, which significant overproductions were detected in our experiments, both are free radicals and reactive oxygen species (ROS). Thus, we may state on the free radicals overproduction in quail embryonic cells as the first step response of the living cells on GSM modulated low intensity MW exposure. These data are in line with the idea on key role of ROS overproduction and oxidative stress induction in mechanisms of adverse biological effects of low intensity microwave/radiofrequency (MW/RF) radiation [8].





**Fig. 5.** The rates of superoxide (a) and nitrogen oxide (b) generation, and level of 8-oxo-dG (c) in cells of 1-day old quail chicks after the exposure to low intensity MW radiation ( $M \pm m$ ,  $n = 5$ ),  $**P < 0.01$ ,  $***P < 0.001$  as compared to the matched controls,  $\#P < 0.05$  as compared to H group;  $###P < 0.01$  as compared to H group;  $####P < 0.001$  as compared to H group. Control – an unprocessed group; GSM – exposed to MW radiation from smartphone (group H), GSM/Waveex – exposed to MW radiation from smartphone + Waveex chip (group H+W)

Significantly increased levels of antioxidant enzymes' activities detected in the exposed 38-h embryos cells is a typical feature of the first stage of the oxidative stress. Next stages of the oxidative stress typically are accompanied by a significant depression of antioxidant enzymes activities. And

the dramatic consequence of the increased levels of  $O_2^{\cdot-}$  and  $NO^{\cdot}$  in the exposed embryonic cells was a pronounced oxidative damage of DNA in cells of one-day old chicks from the MW exposed embryos.

Previously, the level of 8-OH-dG in human spermatozoa was shown to be significantly increased after *in vitro* exposure to low intensity RF radiation (RFR) [24]. Likewise, we demonstrated that the exposure of quail embryos *in ovo* to GSM 900 MHz of  $0.25 \mu W/cm^2$  during a few days of incubation was sufficient for a significant, two-threefold, increase of 8-OH-dG level in embryonic cells [25]. Meanwhile our data obtained on the model of quail embryos may be highly relevant for explanation of wide variety of possible adverse effects of low intensity MW/RF radiation from wireless communication systems on wildlife and ecosystems [5].

It would be logical to assume that most mutagenic effects due to the RFR exposure are caused by oxidative damage to DNA, as the overproduction of ROS in living cells due to WM/RFR exposure was reliably documented. It is known that the most aggressive form of ROS, which is able to affect the DNA molecule directly, is hydroxyl radical [26]. The hydroxyl radicals are generated in cell in Fenton reaction and in Haber-Weiss reaction [27]. On the other hand, increased concentration of NO in addition to superoxide in the RFR exposed living cells can lead to formation of other aggressive form of ROS, peroxynitrite ( $ONOO^{\cdot-}$ ), which can also cause DNA damages [27].

Significant overproduction of ROS leads to oxidative stress in living cells, induces oxidative damages of DNA, and can cause malignant transformation [28]. It is known that in addition to mutagenic effects, ROS play a role as a second messenger for intracellular signaling cascades which can also induce oncogenic transformation [27]. Earlier we hypothesized [25] that low intensity RFR exposure leads to dysfunctions of mitochondria, which results in overproduction of superoxide and NO, and subsequently to ROS-mediated mutagenesis. It is well established that oxidative stress is associated with carcinogenesis. For example, the oxidative stress elicited by Membrane-Type 1 Matrix Metalloproteinase is implicated in both the pathogenesis and progression of prostate cancer [29]. Similarly, a progressive elevation in mitochondrial ROS production (chronic ROS) under hypoxia and/or low glucose level, which leads to stabilization of cells via increased HIF-2 $\alpha$  expression, can eventually result in

malignant transformation [30]. These data together with strong experimental evidence on activation of NADH oxidase under RFR exposure [9] suggest that low intensity RFR is a stress factor for living cell, significant feature of which is oxidative effects and potential carcinogenicity. But again we should underline that the data obtained on the model of bird embryogenesis should not be directly transfer on human biology and only may raise concern and be the incentive for more relevant research as for the risks of particular type of radiation for humans.

On this background, application of Waveex chip for modulation of mobile phone radiation resulted in statistically significant normalizing effect on metabolism in exposed quail embryonic cells. There were detected statistically significant normalization of the rate of somitogenesis, oxidative status of embryonic cells and integrity of DNA in 38-hour quail embryos. Also, statistically significant normalization in superoxide and nitrogen oxide overproductions in tissues of one-day chicks had place. It is important that also statistically significant decrease in oxidative damages of DNA (level of 8-oxo-dG) was detected in this group of chicks as compared to mobile phone MW radiation exposed embryos only. Although indexes of free radicals' generation and oxidative damages of DNA after application of Waveex chip did not return exactly to the control level, they had been much closer to the control levels than indexes in only smartphone radiation exposed group.

It is noticeable that application of Waveex chip to mobile phone did not reveal significant normalizing effect in 10-day exposed embryos, in a period of intensive growth, on disorder in superoxide and nitrogen oxide production due to low intensity MW exposure. But critically important outcome is that even under such strong oxidative stress conditions normalizing effect of Waveex chip on smartphone radiation was detected in a few days, at the end of embryogenesis.

The important outcome of this study in terms of the adverse effects of GSM modulated low intensity MW is that Waveex technology, which according to the developers does not modulate MW but low frequency radiation, nevertheless statistically significantly reduced oxidative and mutagenic effects of smartphone radiation. It obviously raises the question about possible biological effects of low frequency electromagnetic fields from mobile communication devices additionally to MW radiation

effects. The other conclusion should be delivered that while our experiments demonstrated some statistically significant normalizing effects of Waveex chip application, the precautionary principle is topical here in two aspects, as for smartphone radiation itself, and as for Waveex chip practical application.

In conclusion, our experiments on a model of developing quail embryos demonstrated that low intensity MW radiation from a typical commercial smartphone produces statistically significant oxidative and mutagenic effects in quail embryo cells. Application of Waveex chip, which according to the developers modulates low frequency magnetic field from wireless devices, partially but statistically significantly decreased adverse effects of smartphone radiation exposure. Further research on both harmful biological effects of electromagnetic radiation from modern wireless communication devices, and on protective approaches from such effects are needed.

*Conflict of interest.* Authors have completed the Unified Conflicts of Interest form at [http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi\\_disclosure.pdf](http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf) and declare no conflict of interest.

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## ОКСИДАТИВНИЙ ТА МУТАГЕННИЙ ВПЛИВ МІКРОХВИЛЬОВОГО ВИПРОМІНЮВАННЯ НИЗЬКОЇ ІНТЕНСИВНОСТІ НА ЕМБРІОНИ ПЕРЕПЕЛА

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Інтенсивне впровадження систем бездротового зв'язку підняло питання про можливі негативні наслідки антропогенного електромагнітного випромінювання. Метою дослідження було оцінити біологічні ефекти низькоінтенсивного мікрохвильового



випромінювання (МХВ) від типового смартфона Huawei Y51 окремо, або у поєднанні з чіпом Waveex, який врівноважує електромагнітне поле низької частоти, але не впливає на мікрохвильовий сигнал телефону. У дослідженні використано біологічну модель розвитку перепелиних ембріонів *in ovo*. Телефон як джерело випромінювання мікрохвиль низької інтенсивності (0,32 мкВт/см<sup>2</sup>) розміщували на відстані 3 см від поверхні інкубованих ембріонів та активували за допомогою комп'ютерної програми автодозвону (48 с - увімкнено, 12 с - вимкнено). Показано, що опромінення спричиняло статистично значуще збільшення вмісту супероксид аніона, оксиду азоту, ТВА продуктів, порушення цілісності ДНК в клітинах ембріона та підвищення смертності ембріонів. Застосування чіпа Waveex під час впливу частково нормалізувало показники оксидативного статусу та цілісності ДНК в ембріональних клітинах, що вказує на негативний вплив не лише МХВ, а й низькочастотних електромагнітних полів від мобільних пристроїв.

**Ключові слова:** мікрохвильове випромінювання, мобільні пристрої, ембріони перепела, активні форми кисню, ДНК, мутагенні ефекти.

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