SECTION - 3
Effect of Cell-Phone EMFr on Early Development of Chick Embryo

Introduction and Rationale

Evolution of life has taken place under the influence of two universal forces: gravity and electromagnetism. Both play an important role in functional activities of organisms. Ever since introduction of wireless telecommunication in 1990s, the massive phone networking has increased the electromagnetic pollution in cities as well as in the countryside. In recent years, wildlife has been excessively exposed to microwaves and radiofrequency radiations (RFR) signals from various sources which include cell-phones and their towers that are erected indiscriminately without studies of long term effect of radiation on environment and organisms.

Non-ionizing electromagnetic radiation emitted by the cell-phones and towers are a type of pollution of technological origin and are particularly insidious, in that they escape detection by the senses which not only affect humans, but also plants, animals and all living systems. There is growing concern world over of their health effects as cell-telephony is penetrating everywhere.

Objective: To explore the effects of cell-phone EMFr on the early development of chick embryos.

Materials and Methods

Procurement of Material

Fertile hen eggs Chabro (Chandigarh Broiler) for the present study were procured from Central Poultry Breeding Farm, (Govt. of India, Ministry of Agriculture) Industrial Area, Chandigarh.

Experimental Set-up and EMFr Treatment

Thirty eggs were incubated for 24 h in an incubator (Narang Scientific Works, Delhi) at 38±5°C and 50–55% humidity for development. After 23 h of incubation, four
groups (G-II, G-III, G-IV & G-V) each having six eggs (n=6) were exposed to cell-phone electromagnetic field for \(\frac{1}{2}, 1, 2, \) and \(4\) h, respectively (Table 3.1).

Table 3.1. Exposure of various groups of eggs to cell-phone EMF radiations

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of eggs</th>
<th>Exposure time (h)</th>
</tr>
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<tbody>
<tr>
<td>G-I</td>
<td>6</td>
<td>Control</td>
</tr>
<tr>
<td>G-II</td>
<td>6</td>
<td>(\frac{1}{2})</td>
</tr>
<tr>
<td>G-III</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>G-IV</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>G-V</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

Fig. 3.1. A diagrammatic view of the cell-phones positions over eggs.

Fig. 3.2. Exposure of eggs to cell-phone radiations in Exposure Chamber

Eggs (6 nos.) for each group were kept in 3×2 array for exposure. The cell-phones were horizontally placed 2 cm above the eggs for exposure (Figs. 3.1, 3.2). G-I (n=6)
was kept as normal control. This group was not exposed to any type of radiation. Two commercially available GSM Phones (Global Systems for Mobile communication) of 900 MHz frequency band in talk mode were used for exposure. Power density of EMFr exposure was measured with the help of RF Power Density Meter. The average power density was 8.5 μW/cm² (5.7 V/m). Cell-phone’s carrier frequency was 869-905 MHz, modulation frequency 217 Hz, and the maximum average power was 250 mW, maximum peak power 2W and SAR 0.96 W/kg. Experimental area was free of any other source of EMFr except cell-phones used for study. After exposure, eggs of each category were incubated for total 28–30 h period before culmination of experiment. Experiment was repeated thrice and results are expressed as mean±SD of three independent experiments.

Parameters Studied

Age of Embryos

Incubated eggs were opened for examination by preparing a window (Fig. 3.3a,b). Blastodisc was washed in 0.7% hot saline followed by staining in 0.1% neutral red dye. Embryos were observed at ×40 magnification through a Radical Zoom Stereo Trinocular Microscope attached with Nikon (Coolpix-4500) digital camera. Age of the embryos was compared with Hamburger and Hamilton (1951) scale.

Fig. 3.3. Incubated eggs before (a), and after preparation of window exhibiting (b) development of blastodisc, and (c) fully developed embryo.

Morphological Studies

For morphological studies, blastodiscs after incubation of 28–30 h was separated from eggs (Fig. 3.3c). These were washed with 0.7% hot saline followed by distilled water, and then dehydrated in various grades of alcohol (30%, 50%, 70%, 90%, and absolute), and stained with borax carmine (Pearse, 1968). It was observed under ×40
magnification in a Zoom Stereo Trinocular Microscope fitted with a digital imaging system and photographed.

**Histological Studies**

For histological studies, blastodiscs were fixed in Bouin’s, followed by dehydration in different grades of alcohol. Embedding was done in paraffin (Pearse, 1968). Transverse sections of the embryo were cut serially in slices 7μm thick with a rotary microtome and following method was used for present study:

- **Blastodisc fixed in Bouin’s fixative (3–4h)**
- **Washed in hot saline water (0.7%) (x3)**
- **Washed in distilled water (x3)**
- **Dehydrated in different grades of alcohol**
  - 30% (1h), 50% (1h), 70% (washed and left overnight)
- **Dehydrated in 90% (1–2h)**
- **Absolute Alcohol (2h)**
- **Benzene 3:1 (30min), 2:1 (30min), 1:1 (30 min), 1:2 (30min), 1:3 (30min), pure benzene (30min)**
- **Benzene + wax 1:1 (1h)**
- **Saturated with wax at 58–60°C (overnight)**
- **Blastodisc embedded in paraffin wax (58–60°C)**
- **Wax blocks were prepared and fixed on iron stubbs**
- **Sections (5-7 μ) were cut with rotary microtome**
Staining Procedure

- To study histology, sections were stretched in hot water on albumin coated slides and stained with Haematoxylin/Eosin Technique (H/E) (Baker, 1945).
- Dewaxed Bouin’s fixed paraffin section of tissue in two changes of xylene.
- Downgraded the section through various grades of alcohol to water 100% (3 min), 90% (3 min) 70% (2 min), 50% (2 min), 30% (2 min), and water (2 min).
- Sections were stained in haematoxylin for 15–20 min and kept under running tap water for 15 min till sections turn pink.
- Tissues were differentiated in acid water and ammonia water (1–2 dips in each).
- Upgraded up to 90% alcohol by dehydrating the slides 30% (4 min), 50% (4 min), 70% (4 min), and 90% (4 min).
- Stained with eosin (30–60sec), 90% (10 min), 100% (10 min), xylene (10 min).
- Mounted in DPX and observed under suitable magnifications using Phase contrast microscope fitted with a ProRes (Jenoptik-Germany) digital camera.

Results

Five groups (G-I to G-V) each having six eggs were used for one experiment. G-I group (normal control group) was not exposed to any cell-phone radiation. G-II, G-III, G-IV and G-V groups were exposed to cell-phone EMFr for ½, 1, 2 and 4 h, respectively as explained in the materials and methods. Following observations were recorded regarding mortality in various groups after 28 h of incubation.

Fig. 3.4. Percent mortality (±SD) of various groups (G-I to G-V) of eggs of chick embryo 28–30 h after incubation.
Mortality of embryo was higher in exposed groups as compared to control. Maximum mortality was observed in G–V (33±.33%) followed by G–IV (25±.57%). Groups G–III (16.7±.33%) and G–II (16.7±.33) had similar percentage of mortality, whereas, G–I group had only 8.33±0.3% mortality (Fig. 3.4).

**Morphological Studies**

**G-I:** Whole mount (WM) of incubated chick blastodiscs stained in borax carmine showed normal development (Fig. 3.5a,b) as reported in 28–30 h incubated chick embryo. Area pellucida and area opaca are clearly demarcated. Development of embryo in area pellucida is normal. Development of proamnion is visible. Differentiation of head into prosencephalon, mesencephalon and telencephalon is visible. Initiation of optical vesicle differentiation in prosencephalon is clearly visible. Differentiation of heart has also started. Six pairs of somites are visible. Regression of primitive streak and Henson’s node can be observed. Sinus rhombodalis is properly developed. Age of embryo as recorded in comparison of Hamilton scale is 26-29 h in this group.

**G-II:** In ½ h exposed chick embryo, six pairs of somites have developed, however, it is not distinct as in control. Area opaca and area pellucida are clearly visible. Differentiation of head is not well demarcated as in control. Proamnion development is not proper. Start of optical vesicle differentiation in Prosencephalon has not started. Differentiation of heart has also started. Sinus rhombodalis is more obliterated (Fig. 3.5c, d).

**G-III:** In 1 h exposed group the development of blastodisc does not seem to be normal. (Fig. 3.5e, f) Area opaca and area pellucida are less distinct. Head is less developed. Six mesodermal somites are developed. Demarcation of head into different areas is not distinct. Differentiation of heart from the head is less clear and both appear continuous. Optical vesicle differentiation is not visible. Regression of primitive streak and Henson’s node is further less clear when compared with G-II. Sinus rhombodalis is not seen.

**G-IV:** In 2 h exposed embryo, somites are not clearly visible; they appear more primitive in nature. Area pellucida has thinned to a narrow zone around the embryo.
Head development seems to be abnormal. Heart development is grossly affected as seen in Fig. 3.6 (a, b). Primitive streak regression is not clear. Sinus rhombodalis cannot be seen (Fig. 3.6 a, b).

Fig. 3.5. Whole mount blastodisc stained in borax carmine after 28–30 h incubation in G-I (a-b), G-II (c-d), and G-III (e-f). AE-Anterior End, B-Brain, NT-Neural Tube, S-Somite, AO-Area Opaca, AP-Area Pellucida, HN-Henson’s Node, PS-Primitive Streak, PE-Posterior End, OMV-Omphalomesenteric Vein, BI-Blood Islands, OV-Optic Vesicle, Ps-Prosen Cephalon, H-Heart, TS-Telencephalon, MS-Mesencephalon, SR-Sinus Rhombodalis.
**G-V:** Embryos of the experimental group (4 h exposed) had smaller sizes, and delayed development compared to the control. Brain differentiation is completely hampered and there is no distinction of prosencephalon, mesencephalon, and telencephalon. Optical vesicle differentiation has not started. No development of heart is observed. Somites are abnormal and less in number. Primitive streak is very much underdeveloped and Sinus rhombodalis is not visible (Fig. 3.6 c, d).

Fig. 3.6. Whole mount blastodisc stained in borax carmine after 28–30 h incubation in G-IV (a-b) and G-V (c-d). AE-Anterior End, NT-Neural Tube, S-Somite, AO-Area Opaca, AP-Area Pellucida, HN-Henson’s Node, PS-Primitive Streak, PE-Posterior End.

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**Histological Studies of Control and Exposed Groups**

**G-I:** Haemotoxylin/Eosin stained transverse sections (T.S.) of normal control group blastociscs (G-I) exhibited regular development as seen in whole mounts. Neural plate invagination and neuropore is visible. Folding of neural tube is distinct with columnar cells with clear nuclei. Differentiation of coelom is also distinct. Somite development
is normal. Posterior part of blastodisc consists of normal yolky cells which are used by embryo for its growth. Cell shows dark satin due to presence of yolk inside. Somite differentiation in sections is also distinct (Fig. 3.7a–f).

**Fig 3.7.** Haemotoxylin/Eosin stained T.S. of chick blastodiscs of normal control (G-I) under various magnifications; a (×40), b (×100), c (×1000), d (×100), e (×100), and f (×1000). NP-Neural Plate, Np-Neuropore, C-Coelom, YC-Yolky Cell, Ph-Posterior Hypoblast, CC-Columnar Cell.

**G-II:** Haemotoxylin/Eosin stained transverse sections (T.S.) of chick blastodiscs which were exposed for ½ h exhibited a little hampered development as compared to control group (G-I). Bending of neural tube is not distinct. Columnar cell visible in
control group’s neural plate are not distinct in this group. Differentiation of coelom is also not complete. Neuropore is not visible due to poor invagination of neural plate cells. Posterior half of bastodisc did not contain regular yolk filled cells. It has vesiculated cells with improper cell margins (Fig. 3.8 a–e).

Fig 3.8. Haemotoxylin/Eosin stained T.S. of chick blastodisc of G-II under various magnifications; a (×100), b (×1000), c (×100), d (×400), and e (×400). NP-Neural Plate, Np-Neuropore, C-Coelom, EC-Ectoderm, YC-Yolky Cell, Ph-Posterior Hypoblast.

G—III Embryos which were exposed for 1 h to EMF radiations of cell-phones exhibited more distortions. Haemotoxylin/Eosin stained transverse sections (T.S.) of 1

100
h exposed chick blastodisc revealed very early stage of neural tube differentiation. Neural plate cells are poorly developed and not separated from surrounding cells. Bending of neural plate is not complete as evident in control group. Neither coelom differentiation is visible nor are somites distinct. Posterior half of blastodisc also exhibits disoriented cells with no cell membranes present in between them. Dark granular cells with disorganized cytoplasm are visible (Fig 3.9 a–d).

Fig 3.9. Haemotoxylin/Eosin stained T.S. of chick blastodisc of G-III under various magnification: a (×100), b (×100), c (×1000), d (×100), e (×1000), f (×400). NP-Neural Plate, Np-Neuropore, YC-Yolky Cell.
**G-IV and G-V:** Embryos which were exposed for 2 h (Fig. 3.10 a–d) and 4 h (Fig. 3.11 a–d) exhibited maximum distortion in development of blastodisc. It is evident in whole mount embryo of respective groups too. Disorganization in cells of epiblast and hypoblast is visible. No differentiation of neural plate cells is visible. There is no demarcation between cells of neural plate. Further, characteristic columnar cells are absent.

**Fig 3.10.** Haemotoxylin/Eosin stained T.S. of chick blastodisc of G-IV under various magnifications: a (×100), b (×1000), c (×1000), and d (×1000). **DNP-** Distorted Neural Plate Cells, **YC-** Yolky Cells.

No differentiation of coelom or somites is there. Disorganization of yolk cells present in posterior area of blastodisc is also visible. Cells are distorted and loosely attached. Dark granulated yolk cells are not visible (Fig. 3.10 a–d and 3.11 a–d).
From the present experiment, it can be concluded that distortion of cells is incorporated with exposure to EMF of cell-phones. This distortion in development increases with increase in exposure time as evident from observations.

Fig. 3.11. Haemotoxylin/Eosin stained T.S. of chick blastodiscs of G-V under various magnifications, a (×100), b (×100), c (×400), d (×400). NP-Neural Plate, YC-Yolky Cells.

Discussion

Toxic effects of the chemicals are well known which are based on stage of cell/organism. Similarly, toxic effects of radiations on different systems are also being evaluated. For example radiofrequency and microwaves EMF\textsubscript{r} emitted from cell-phone are found to be toxic and cause genotoxic effects on rats (Lai and Singh, 1995, 1996). The present study points to greater mortality in chick embryos which were exposed to EMF radiations of cell-phones. These observations are in agreement with earlier studies of Youbicier \textit{et al.} (2000) who reported that eggs exposed to GSM mobile phone and incubated for 21 days had greater mortality. Grigorev (2003)
demonstrated that GSM mobile phone exposure for 21 days increased chick embryo mortality to 75%.

Further, the cell-phone radiations hampered early growth of chick embryo. It induced faulty neural plate bending, distorted coelem development, and hampered differentiation of somites and hypoblast in exposed groups. The extent of damage increased with increase in exposure time. However, there have been contrasting reports in this regard. Earlier, Farrell et al. (1997) reported abnormal developmental changes in chick embryo upon exposure to weak magnetic fields of 60 Hz, ~1 μT. On the contrary, Martin (1992) reported no significant malformation in chick embryos in response to 60 Hz with 3 μT magnetic fields. Likewise, Veicsteinas et al. (1998) reported that exposure of low frequency 50 Hz intermittent with 200 μT magnetic field did not cause developmental abnormalities in chick embryos. An effects of EMF are frequency based, and depends upon dose (intensity of EMF), duration (time of exposure) and distance (how far from source of exposure).

The observations made in present study are parallel by earlier report that cell-phone radiations (900 MHz) increased mortality and tissue damage in developing chick embryo (Ingole and Gosh, 2006). In the present study, no brain differentiation occurred in embryos exposed to EMF for 1h to 4h. Histologically, the exposed chick embryos exhibited distortion of neural plate cells and absence of coelom differentiation. Earlier such an effect of EMF on brain development, particularly telencephalon has been reported (Lahijani et al., 2007). Recently, cell-phone in call mode has been reported to affect eye differentiation in chick embryo (Zareen et al., 2009). However, it is for the first time that cell-phones in talk mode have been reported to affect early neural differentiation of exposed chick embryos. Radiation level is higher in talk mode as compared to call mode.

Not only has the developmental changes, even the activities of certain enzymes involved in embryo development been hampered by EMF. EMF exposure induces quick and chronic effects due to increased free radical levels (Simko & Mattsson, 2004). A short term exposure results in phagocytosis and leading to free radical production and enhancement in life time of these radicals. Generation of free radicals damage the membrane leading to generating of various ill effects of EMF. IARC has
clarified EMF as possible carcinogenic also (IARC, 2002). For example, EMF has been reported to decline the activity of ornithine decarboxylase in EMF exposed embryo leading to morphological abnormalities (Farell et al., 1997). In contrast, the activity of enzyme glutamine synthetases involved in developmental processes has also been reported to increase in EMF exposed embryo (Rajendra et al., 2004).