ORIGINAL ARTICLE
Effect of Exposure to Electromagnetic Radiation on Sex Steroids and Systemic & Local Uterine Redox Status during Early and Late Pregnancy in Rats

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ABSTRACT
Background: pregnant women are at high risk of exposure to environmental EMR because of their higher oxygen consumption and amniotic fluid-induced ROS, which is associated with adverse pregnancy outcomes. The present study was designed to demonstrate the effects of EMR exposure at 900, 1800, and 2450 MHz on the female sex hormones (estrogen, progesterone) and systemic and local uterine redox status during early and late stages of pregnancy in adult albino rats.

Methods: The present study was carried out on 88 weaned albino female rats were divided into four equal groups: control group: rats were not exposed to EMR, group2: rats were exposed to 900 MHz EMR, group3: rats were exposed to 1800 MHz EMR (emitted from mobile phones), and group 4: rats were exposed to 2450 MHz EMR (emitted from Wi-Fi). After induction of pregnancy each group was subdivided into two equal groups: early pregnant and late pregnant subgroups. At the end of each experimental period samples were collected for estimation of serum estradiol & progesterone and plasma and local oxidative stress markers.

Results: The present study showed that EMR exerted a significant frequency and duration dependent reduction in serum estrogen, progesterone, plasma TAS, vitamin C and uterine GSH which was accompanied by significant increase in plasma and uterine MDA.

Conclusions: Exposure to EMR induced progressive reduction in sex steroid hormones, and imbalance in oxidative/antioxidative stress parameters in pregnant rats. These effects were dependent on the frequency and the duration of the EMR.

Key word: EMR; Oxidative stress; Pregnancy.

INTRODUCTION
The wireless local area network (WLAN) systems, a substitute to wired internet access provides a mean of communication and information exchange; but it produces electromagnetic radiation (EMR) at a frequency of 2450 MHz. Also mobile phones produce EMR through their base station antennae in the frequency range of 900–1800 MHz for the Global System for Mobile Communications [1].

The portable computers and mobile phones are commonly placed on the legs and in the pockets of humans, and thus expose the genital area to EMR [2].

Reactive Oxygen Species (ROS) production is associated with enhanced exposure to EMR [3] and together with free radicals affect the reproductive system in both humans and animals [4].

On the other hand, pregnant women are at specific risk of exposure to environmental EMR because of their higher oxygen consumption and amniotic fluid-induced ROS production [5].

There are some data about the effects of extremely low electrical field (ELF)-EMR (50 Hz) on the female reproductive function [6].

So the aim of the present study is to investigate the effects of exposure to different waves of EMR (900, 1800 and 2450 MHz) on sex steroids and the systemic & local uterine redox status during early and late pregnancy after
MATERIAL AND METHODS
The study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. Experiments complied with the ARRIVE guidelines and was carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

The present study was carried on 99 weaned albino rats 88 female rats plus 11 male rats for fertilization weighing 35-50 gm (initial weight).

The animals were divided into male and female groups:
- **Male group (n=11):** used for induction of pregnancy that was carried out at the age of 8 weeks in both genders.
- **Female groups (n=88):** Female rats were subdivided according to EMR exposure into 4 equal subgroups: Subgroup 1 (control), Subgroup 2 (900MHz), Subgroup 3 (1800MHz) and Subgroup 4 (2450MHz).

Female rats in control subgroup were not exposed to EMR and after induction of pregnancy; it further subdivided into 2 equal subgroups early (1a) at 7th and late (1b) at 18th days of gestation.

Female rats in subgroups 2, 3 and 4 were exposed to different frequencies of EMR every day from age of 3 weeks and after induction of pregnancy; each subgroup further subdivided into 2 equal subgroups: early pregnant subgroups were exposed until 7th day of gestation (2a, 3a and 4a) and late pregnant subgroups were exposed until 18th day of gestation (2b, 3b and 4b).

At the previous selected days of pregnancies all pregnant subgroups were sacrificed and blood samples were collected for measurement of serum estradiol & progesterone and measurement of plasma oxidative stress markers (lipid peroxidation and reduced glutathione).

**Induction of pregnancy:**
Vaginal smears taken from the female rats were examined daily by using light microscope to ensure that they were in regular estrus cycle. The estrus phase of the estrus cycle was detected by the presence of cornified epithelial cells which increase in number and eventually predominate as the estrus progressed [7]. The female proved to be in estrus phase was paired with a mature male rat in a separate cage for 24 hours. After mating, females were subsequently isolated until the time of analysis to ensure accurate gestation timing, and in the next morning a vaginal smear taken. Copulation was confirmed by the presence of a copulation plug or spermatozoa in the vagina. The presence of sperms or vaginal plug was designated gestational day zero [8].

**Sampling of blood:**
Blood samples were collected from the retro orbital plexus of each rat under ether anesthesia using glass capillaries. The collected blood used for analysis of plasma MDA, TAS and vitamin C. It is also used for analysis of serum estradiol and progesterone.

**Preparation of uterine tissue samples:**
The rats were sacrificed on 7th and 18th days of gestation in each group by decapitation. The abdomen was opened, the uterine horns were dissected, and then the pregnant uteri were cleaned from fat, placenta, fetus, fetal membrane and then rinsed thoroughly with cold saline solution and after processing used for assaying MDA and GSH.

**Assessment of lipid peroxidation:**
Lipid peroxidation levels in the uterine homogenate and plasma samples were measured by assaying for thiobarbituric acid-reaction substances [9].

**TAS analysis:**
The plasma TAS level was measured using Colorimetric Assay kits. The results in the plasma were expressed in 1 mol H$_2$O$_2$ equivalent/liter (1 mol H$_2$O$_2$ equiv/l).

**Measurement of plasma vitamin C:**
Quantification of ascorbic acid in the plasma samples was performed according to the method of Rutkowski and Grzegorczyk [10] the absorbance of the samples was measured spectrophotometrically at 700nm.

**Measurement of reduced glutathione:**

The GSH content of the uterine samples was measured at 412 nm using Ellman’s reagent [11].

**Estimation of serum Estradiol (E2) level:** using rat ELISA Kits.

**Estimation of serum Progesterone level:** using rat ELISA Kits.

**Statistical analysis:**

SPSS version 18.0 program for Windows (SPSS Inc. Chicago, IL, USA) was used.

**RESULTS**

It was found that all studied frequencies of EMR (900, 1800 and 2450 MHz) had significantly decreased serum E2 and progesterone in early and late pregnant rats (p<0.001) when compared to control groups (Tables 1 and 2) (Figure1).

Moreover, exposure to EMR with frequency of 2450 MHz significantly decreased serum E2 in early (p<0.05) and late (p<0.001) pregnant rats when compared to early and late pregnant rats exposed to EMR with frequency of 900 MHz in addition, serum E2 was significantly decreased in late pregnant rats exposed to 2450 MHz (group 4b) when compared to rats exposed to 1800 MHz (group 3b) frequency radiation (p<0.01). However, there was an insignificant change in serum E2 among early and late pregnant groups exposed to radiation with frequency of 900 and 1800 MHz (p>0.05) (Tables 1 and 2).

Regarding serum progesterone, it showed significant reduction in both early and late pregnant exposed to 1800MHz (Tables 1 and 2) (Figure1).

Moreover, our results showed significant progressive increase in serum MDA with increase the frequency of the EMR in early and late pregnant rats. It was significantly high in early pregnant animals in comparison to control (p< 0.05, 0.001 and 0.001; respectively). Moreover, in late pregnant it showed significant increase in all exposure groups in comparison to control group (P<0.001, 0.05, 0.001; respectively) (Tables 1 and 2) (Figure2).

In addition, its level showed significant increase in early and late exposed to 1800 MHz frequency (p< 0.05; in both groups) when compared to groups exposed to 900MHz. Furthermore, rats exposed to 2450 MHz showed significant increase in its level in comparison to those exposed to 900MHz (p<0.001; in early and late pregnancy groups) and 1800MHz (p<0.001; in early and late pregnancy groups) (Tables 1 and 2).

As regard to uterine MDA, it showed significant increase in early and late exposure groups exposed to 900 MHz (p<0.05; 0.05 respectively), 1800 MHz (p<0.05; 0.01 respectively) and 2450 MHz (p<0.01, 0.001; respectively) when compared to control group.

In addition, exposure to 1800 MHz in late pregnant rats significantly increased uterine MDA (p<0.05) in comparison to group 2b (table 2), with insignificant change between both groups in early pregnant rats (p>0.05) (Table 1).

Moreover, early and late exposure to 2450 MHz showed significant increase in its level in comparison to those exposed to 900MHz (p<0.05, 0.01; respectively) and 1800MHZ (p<0.05; in early and late pregnancy groups) (Tables 1 and 2).

However, a non-significant difference in the uterine GHS level in early pregnant rats exposed to 900 MHz radiation in comparison to control group (p>0.05). In contrast, late pregnant rats exposed to 900 MHz radiation showed significant increase in uterine GSH level in comparison to control group (p<0.05).
However, there was an insignificant difference in uterine GSH levels among groups exposed to 900 MHz and 1800 MHz radiation.

Moreover, its level showed significant increase in early and late exposed to 1800 MHz frequency (p<0.05; 0.01; respectively) when compared to control group. Furthermore, rats exposed to 2450 MHz showed significant increase in its level in comparison to control group (P<0.001; in early and late pregnancy groups), those exposed to 900 MHz (p<0.001; in early and late pregnancy groups) and those exposed to 1800 MHz (p<0.05; in early and late pregnancy groups) (Tables 1 and 2).

In respect to the antioxidant markers, plasma TAS showed significant decrease in all exposure groups with p<0.05 in early and late pregnant rats exposed to 900 MHz radiation, p<0.05 and p<0.01 in early and late pregnant rats exposed to 1800 MHz radiation; respectively, and p<0.01 and p<0.001 in early and late pregnant rats exposed to 2450 MHz radiation; respectively when compared to control group. (Figure 3)

Moreover, early and late exposure to EMR with 1800 MHz frequency significantly reduced plasma TAS when compared to groups exposed to 900 MHz (p<0.05, 0.01; respectively). Further, early and late exposure to EMR with 2450 MHz frequency significantly reduced plasma TAS when compared to groups exposed to 900 MHz (p<0.01, 0.05; respectively), and in comparison to groups exposed to 1800 MHz (p<0.01) (Tables 1 and 2) (Figure 3).

Interestingly, plasma vitamin C level showed a significant reduction in early and late groups exposed to 1800 MHz (p<0.05, 0.01; respectively) and 2450 MHz radiation (p<0.01, 0.001; respectively) in comparison to control group. However, no significant difference was found between group 2a and control group (p>0.05) (Figure 4)

Moreover, early and late exposure to 2450 MHz significantly reduced vitamin C level in comparison to rats exposed to 900 MHz (p<0.01, 0.05; respectively). Furthermore, late exposure to 2450 MHz significantly reduced vitamin C level in comparison to rats exposed 1800 MHz (p<0.05). In contrast, there was non-significant change in vitamin C level between both groups in early pregnant rats (p>0.05) (Figure 4).

Table 1. Effect of EMR on all studied parameters in the early pregnant studied subgroups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups(n=11)</th>
<th>Group 1a (Control)</th>
<th>Group 2a (900MHz)</th>
<th>Group 3a (1800MHz)</th>
<th>Group 4a (2450MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>serum estradiol (pg /ml)</td>
<td>54.76±5.01</td>
<td>47.40±6.20</td>
<td>45.66±2.94</td>
<td>42.65±2.77</td>
<td></td>
</tr>
<tr>
<td>serum progesterone (pg/ ml)</td>
<td>36.94±3.79</td>
<td>29.72±2.54</td>
<td>25.46±2.62</td>
<td>22.72±2.41</td>
<td></td>
</tr>
<tr>
<td>Plasma MDA (nmol/L)</td>
<td>112.57±6.04</td>
<td>121.77±4.31</td>
<td>131.71±9.06</td>
<td>168.17±8.41</td>
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</tr>
<tr>
<td>Plasma TAS (µmol H2O2 equiv. /L)</td>
<td>2.49±0.11</td>
<td>2.29±0.12</td>
<td>2.15±0.09</td>
<td>1.74±0.14</td>
<td></td>
</tr>
<tr>
<td>Plasma vitamin C (µmol/L)</td>
<td>40.36±5.38</td>
<td>38.32±4.69</td>
<td>35.34±5.54</td>
<td>32.97±5.69</td>
<td></td>
</tr>
<tr>
<td>Uterine MDA (nmol/gm tissue)</td>
<td>6.86±0.37</td>
<td>7.42±0.67</td>
<td>7.68±0.74</td>
<td>8.25±0.65</td>
<td></td>
</tr>
<tr>
<td>Uterine GSH (µmol/gm tissue)</td>
<td>28.62±3.21</td>
<td>26.56±2.47</td>
<td>25.70±2.66</td>
<td>23.01±3.19</td>
<td></td>
</tr>
</tbody>
</table>

Values are means± SD

a: sig vs control group; b: sig vs group2a; c: sig vs group 3a; *: p<0.001; $: p<0.01; #p<0.05
Table 2. Effect of EMR on all studied parameters in the late pregnant studied subgroups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1b (Control)</th>
<th>Group 2b (900MHz)</th>
<th>Group 3b (1800MHz)</th>
<th>Group 4b (2450MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum estradiol (pg/ml)</td>
<td>91.82±7.02</td>
<td>72.59±6.43</td>
<td>71.15±6.92</td>
<td>61.62±4.94</td>
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<tr>
<td>Serum progesterone (pg/ml)</td>
<td>33.24±3.09</td>
<td>27.87±2.62</td>
<td>25.24±2.80</td>
<td>20.92±3.22</td>
</tr>
<tr>
<td>Plasma MDA (nmol/L)</td>
<td>113.02±6.43</td>
<td>127.76±9.08</td>
<td>137.38±11.42</td>
<td>185.04±7.14</td>
</tr>
<tr>
<td>Plasma TAS (µmol H₂O₂ equiv./L)</td>
<td>2.53±0.15</td>
<td>2.22±0.11</td>
<td>2.08±0.14</td>
<td>1.63±0.22</td>
</tr>
<tr>
<td>Plasma vitamin C (µmol/L)</td>
<td>39.96±3.36</td>
<td>35.77±4.90</td>
<td>33.78±6.23</td>
<td>28.64±4.43</td>
</tr>
<tr>
<td>Uterine MDA (nmol/gm tissue)</td>
<td>6.89±0.49</td>
<td>7.59±0.48</td>
<td>8.18±0.61</td>
<td>9.20±0.82</td>
</tr>
<tr>
<td>Uterine GSH (µmol/gm tissue)</td>
<td>29.18±4.61</td>
<td>24.98±3.03</td>
<td>23.67±3.61</td>
<td>20.28±3.52</td>
</tr>
</tbody>
</table>

Values are means± SD
a: sig vs control group; b: sig vs group2b; c: sig vs group 3b *: p<0.001, $: p<0.01; #: p<0.05

Figure 1. Serum estradiol and progesterone levels in early and late pregnant subgroups
Figure 2. Plasma MDA levels in early and late pregnant subgroups

Figure 3. Plasma TAS levels in early and late pregnant subgroups
DISCUSSION

There is growing interest in the increase risk of EMR-induced environmental pollution. EMR exposure modifies cellular antioxidant levels as well as hormonal homeostasis in humans and animals. Although the pathophysiologic mechanisms that are responsible for such effects remain unknown, common theories include changes in temperature, membrane permeability, and ROS production may be implicated [12]. The current reports about the effect of EMR exposure on female reproductive tract in human [1] and animals [13] are conflicting. Although in most cases, exposure to EMR increases ROS levels in human, mouse and rat cells, there are also studies showing that ROS levels were decreased or not affected by EMR. Multiple factors could cause these discrepancies, including EMR type, intensity, frequency and exposure time [14].

The results of the current study showed significant increases in the systemic and local lipid peroxidation levels (MDA) associated with significant decreases in systemic (TAS and vitamin C) and local (GSH) antioxidant markers in both early and late pregnant subgroups after exposure to EMR at different frequencies (900, 1800, and 2450 MHz) compared to the control subgroups. Oxidative stress implicates in the etiopathogenesis of local female reproductive diseases such as endometriosis, polycystic ovarian disease, hydatidiform mole, and unexplained infertility. Also, there is growing evidence on its involvement in the pathophysiology of pre-eclampsia, free induced birth defects and other situations such as abortions [4]. In addition, oxidative stress may induce luteal regression and insufficient luteal hormonal support for the continuation of a pregnancy [15].

This present results are in agreement with several studies which reported changes in redox status in the different tissues after exposure to different frequencies of EMR. As, Türedi et al., [16] who observed that continuous exposure to 900-MHz of EMR for 1 h / day on postnatal
days 22-59, caused an increase in oxidative stress and various pathological changes in bladders and kidneys of male rats. Also, Bodera et al., [17] revealed that exposure to 1800 MHz of EMR caused elevation of MDA levels in blood, brain, and kidney of the experimental rats.

Furthermore, other studies detected marked increases in the inflammatory oxidative stress markers after exposure to EMR at 2450MHz exposures on different organs and tissues like heart [18], laryngeotracheal mucosal tissues [19], liver and kidney [20] by increasing MDA, total oxidant status, GSH-PX levels and decreasing TAS levels.

Moreover in concordance to the current data, Aksen et al., [21] stated that prolonged exposure to 50 Hz induced increasing in MDA levels in rat uterus and ovaries. Also Yuksel et al., [22] and Shahin et al. [23] who demonstrated that mobile phones emitting 900 and 1800 MHz and Wi-Fi emitting 2450 MHz EMR induced modification in the concentrations of local uterine oxidative stress markers of growing and maternal rats associated with drop in the plasma concentration of vitamin C, E and A.

On the other hand, in contrast to the present results Kismali et al., [13] detected non-significant difference of oxidative stress markers in the blood of pregnant rabbits after EMR exposure. The differences between the pervious study and the present study may be due to differences in: 1) species; rabbit versus rats, 2) duration of exposure; 7days versus 6 to 7 weeks, 3) frequencies of EMR; 1800 MHz versus 900, 1800 and 2450 MHz respectively.

In addition the current results showed significant decreases in the serum estradiol and progesterone levels of both early and late pregnant subgroups after chronic exposure to EMR (900, 1800 and 2450 MHz) when compared with the control subgroups. These results are in agreement with Ozguner et al., [24] who demonstrated that exposure to EMR at 900 MHz declines plasma FSH & LH levels in rats. Moreover, Shi et al., [25] and Akhras [26] reported that the levels of FSH, LH, estrogen and progesterone were significantly decreased after exposure of the gravid and non-gravid rats to ELF of 50 Hz EMR.

Furthermore these findings are confirmed by the results obtained from Yuksel et al., [22] and Shahin et al. [23] who detected that exposure to 900, 1800 and 2450 MHz EMR causes decrease in FSH, LH, estrogen, progesterone and prolactin levels in maternal and growing rats.

In contradiction to the above results, it was reported that the plasma progesterone and estrogen levels in rats were not changed by ELF (50Hz) induced EMR exposure [27]. The discrepancies between the present finding and the latter study may be explained by difference in frequency of EMR 900, 1800 and 2450 MHz versus 50 Hz respectively.

So, the significant shift of the systemic and local redox balance to the oxidative side in the early and late pregnancies after chronic exposure to different EMR frequencies (900, 1800 and 2450 MHz) , starting before and during gestation in the present study pointed to the direct injurious effects of emitted mobile and Wi-Fi EMR waves on the uterus. Further investigations are needed in humans, especially on young teenagers who often use their mobile phones for several hours per day.

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

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