Effect of Mobile Phone on Male Fertility in Rats
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ABSTRACT
The study was conducted to evaluate the effect of 900MHz radiofrequency – electromagnetic waves (RF-EMW) sent out from mobile phone on rats male fertility. 36 male wistar rats 12-week aged (300-400 g) body weight were used, divided into three groups, control group, 1h/day for 30 days and 2h/day for 30 days exposure groups and then divided into A and B subgroups after end of experiment. A subgroups rats were used for semen analysis and sex organs weights were measured, also hormonal analysis were done. B subgroups rats were used for fertility test. The results show a significant decrease in testis weight in 1h/day exposure group for 30 days, a significant decrease in prostate weight in 1h/day, 2h/day for 30 days exposure groups when compared with control group, an increase in seminal vesicle weight in 2h/day group was significant as compared with control group and, A highly significant decrease in number of total sperm count in a both exposure groups verses control group. The percentage of live sperms was also affected as highly significant decrease of living sperms when compared to control groups in exposure groups during the experiment. There are a highly significant increase in percentage of abnormal sperms in both exposure groups as compared to control group. There are a significant increase in mixing days until parturition and a decrease in number of pups in both exposure groups as compared to control group. In conclusion the electromagnetic waves emitted from the mobile phone had a hazard effects on fertility of male rats.

INTRODUCTION
Interesting about the possible hazard effects of mobile phone usage are growing as the number of users has increased tremendously over the past years. Mobile phone technology uses radiofrequency electromagnetic radiation (RF-EMR) and has drastically increased the RF-EMR exposure encountered in daily life. (Mailankot et al., 2009). Cell phone has become indispensable devices in our daily life, these phones operates between 400MHz and 2000MHz frequency bands and emit radiofrequency electromagnetic waves (EMW). (Agarwal et al., 2008). These phones operate at different frequencies, differing in respect to the frequency usage in different countries. Concerns are growing about the possible hazard effects of radio-frequency electromagnetic waves (RF-EMW) emitted by these devices on human health. (Agarwal, 2007). The effects of EMW on living organisms depends on the wave frequency and intensity, The hazardous effect of radio waves of high frequency is associated with an increase in body temperature. (Wdowiak et al., 2007). The problem of the lack of offspring is a phenomenon concerning approximately 15% of married couples in Poland, Infertility is defined as
inability to conceive after a year of sexual intercourse without the use of contraceptive


(Wdowiak et al., 2007). The causative factors may be chemical substances, ionizing radiation, stress, as well as electromagnetic waves (Petrelli and Mantovani, 2002). Males are exposed to the effect of various environmental factors which may decrease their reproductive capabilities (Claman 2004), (Sheiner et al., 2003). Significant decreasing in concentration, total volume of semen with no obvious cause of the deteriorated semen parameters defined as idiopathic infertility. (Fejes et al., 2007), (Carlesen, et al., 1992). Idiopathic infertility is defined as decreased sperm quality with no organic, genetic, endocrine alteration in the background and no infection affecting genital system. (Fejes et al., 2005). Various environmental factors, such as heat, chemical agents, pullets and radiation like X-rays, RF-EMR from mobile phone (GSM) General System for Mobile communication with radiofrequency 800 – 900 MHz and 1.8 – 2.2 GHz is another type of radiation are affect genital system. (Aniolczyk, 1999). Recent years have been a very rapid development in cellular telecommunications based on the emission of EMW, the effect of mobile phone on health has not yet been fully confirmed. It should be wrong to ignore the question whether such wide use of mobile phone has a side effect on living organisms especially reproductive health, so the aim of this study is to evaluate the effect of 900MHz RF-EMW from mobile phone on rats male fertility.

MATERIALS AND METHODS

Thirty six male wistar rats 12- week aged (300 -400 g body weight) and 54 adult female rats were obtained from laboratory animal house in veterinary medicine college of Mosul university. The male rats were divided in three groups, control group (n= 12), exposure group 1h/day for 30 days (n= 12) and exposure group 2h/day for 30 days (n= 12). Male rats were exposed continuously to mobile phone 900MHz frequency (using automatic redial mode). Rats were placed in Plexiglas cages (60 x 30 x 20 cm) with drilled ventilation hole 2.5 cm in diameter, which has been attached with mobile phone hand set. Control group prepared for the mobile phone exposure in switch off mode. Rats housed in a plastic cages under controlled temperature and light, food and tap water were freely available through out the experiment. Nokia N70 mobile phone (nokia corporation – Finland) was used in this experiment. Body weight of rats in all groups were measured before and after experiment. All groups were divided into 2 subgroups after end experiment each of which is contain 6 male rats (A and B subgroups). Subgroup A rats were used for hormonal assays, semen analysis and measurement sex organ weights. Subgroup B rats were used for fertility test.

Hormonal analysis:- Blood samples were taken from rat eyes using a capillary tubes and collecting blood in a plane tubes (without anti-coagulants) to obtain serum for measuring Testosterone, luteinizing hormone (LH) and
follicle stimulating hormone (FSH) levels, ELISA Test for the quantitative determination, (Human Gesellschaft für Biochemica und Diagnostica mbH Germany). Blood samples were separated by centrifugation at 10000 rpm for 15 minutes and serum was stored at -18°C until use.

**Sex organ weights**: - sex organs weight which include the testis, epididymis, seminal vesicle and prostate were recorded by using Sartorius "BL210S" balance at the end of experiment.

**Determination of total sperms count**: - The caudal epididymis was further cut to release all sperms in sperm collection vial containing 5ml BSA Hanks solution and the fluid in the vial was filtered through a nylon-mesh sieve. The resulting stock filtered was then diluted with formalinized saline (0.1 ml of filtrate in 1.9 ml saline) and the number of sperms was determined with hemocytometer. The measured sperm number was multiplied by the dilution factor to yield the total sperm count. (Mailankot et al., 2009)

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\text{Sperm count} = \left( \frac{\text{counted sperms}}{80} \right) \times 4000 \times 1000
\]

**Determination of live and dead sperms**: - Using the epididymis head in this procedure by cutting the head in 2 ml normal saline in Petri dish to release all sperms. Mixing 1 drop of semen mixture with 2 drops of eosin–negrosin stain on slide and making a thin film on other slide (Noakes and Parkison, 2001), percentage of live, dead and abnormal sperms were measured.

**Fertility test**: - Control, 1h/day exposure for 30 days and 2h/day exposure for 30 days subgroups, male rats was mixed with normal adult female rats in separated Plexiglas cages (each cage has 1 male with 3 females). After 15 days of mixing, every three days females were checked for pregnancy (by checking abdominal size) and separate pregnant females until parturition in especial cages.

Following parameters were observed:
1. Determining the days from mixing males with females until parturition.
2. Determining number of pups in each parturition.
3. Determining the pregnancy percentage.

**Statistical analysis**
All data were presented as Mean ± S.E and analyzed via SPSS version 11.5 software using ONE-WAY ANOVA LSD test, Chi-square test. The significance level in comparisons was considered to be \(p \leq 0.05\).

**RESULTS AND DISCUSSION**
The effect of radiofrequency electromagnetic waves (RF-EMW) emitted from mobile phone causes significant decrease in testis weight (\(p \leq 0.001\)) in 1h/day exposure for 30 days group as compared with control group, also cause a significant decrease in prostate weight (\(p \leq 0.05\)) in 1h/day exposure, (\(p \leq 0.01\)) in 2h/day exposure for 30 days groups as compared with control group, a significant (\(p \leq 0.05\)) increase in seminal vesicle weight in 2h/day exposure group as compared with control group, (Table 1). RF-EMW causes decrease in both testis and prostate weight perhaps due to thermal effect of mobile phone (which generates heat when be on redial mode) on these tissues.
and may be due to the effect of oxidative stress happens in testis and prostate causing atrophy in their tissues, due to increase the amount of radiofrequency energy absorbed into these tissues that emitted from mobile phone, many investigations fined this effect (Mailankot et al., 2009), (Wdowiak et al., 2007) and (Agarwal et al., 2008). The increase in seminal vesicle weight happened still understand.

Table (1) Effect of 900MHz RF-EMW from mobile phone on sex organs weights

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Testis weight mg/100g B.W</th>
<th>Epididymis weight mg/100g B.W</th>
<th>Prostate weight mg/100g B.W</th>
<th>Seminal vesicle weight mg/100g B.W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>365.5 ± 7.7</td>
<td>132 ± 5.5</td>
<td>569.5 ± 62.9</td>
<td>143 ± 8.2</td>
</tr>
<tr>
<td>1h/day exposure for 30 days</td>
<td>292.8 ± 34.1*</td>
<td>129.5 ± 11</td>
<td>422 ± 47.2*</td>
<td>171.1 ± 14.2*</td>
</tr>
<tr>
<td>2h/day exposure for 30 days</td>
<td>308.8 ± 10.7</td>
<td>113.6 ± 3.9</td>
<td>371 ± 31.2**</td>
<td>177.3 ± 5.7*</td>
</tr>
</tbody>
</table>

* mean values significant at p ≤ 0.05 , ** mean values significant at p ≤ 0.01 , Values = mean ± SE.

There was a significant increase in percentage of abnormal sperms in both exposure groups as compared to control group (Table 2).

Table (2) Effect of 900MHz RF-EMW from mobile phone on sperm parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sperm count X10⁶/ml</th>
<th>Live sperms %</th>
<th>Abnormal sperms %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1658.3 ± 161.9</td>
<td>94.16 ± 1.3</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>1h/day exposure for 30 days</td>
<td>687.5 ± 79.5***</td>
<td>54.5 ± 1.4***</td>
<td>22.5 ± 2.2***</td>
</tr>
<tr>
<td>2h/day exposure for 30 days</td>
<td>754 ± 37.8***</td>
<td>39.16 ± 1.1***</td>
<td>28.3 ± 2.5***</td>
</tr>
</tbody>
</table>

*** mean highly significant p ≤ 0.001 , Values = mean ± SE.

There is a significant decrease in serum testosterone level in both exposure groups (p ≤ 0.05) as compared with control group, with significant (p ≤ 0.05) decrease in serum FSH level in 2h/day exposure for 30 days group as
compared with control, serum LH levels in all groups show no statistically differences, (Table 3).

A significant ($p \leq 0.001$) decrease in number of total sperm count and the live sperms percentage also affected as very significant ($p \leq 0.001$) decrease of living sperms in both exposure groups versus control group during the experiment.
Table (3) Effect of 900MHz RF-EMW from mobile phone on sex hormones

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Testosterone (T) Pg/ml</th>
<th>luteinizing hormone (LH) m IU/ml</th>
<th>follicle stimulating hormone (FSH) m IU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.65 ± 0.02</td>
<td>2.51 ± 0.27</td>
<td>3.85 ± 0.18</td>
</tr>
<tr>
<td>1h/day exposure for 30 days</td>
<td></td>
<td>0.32 ± 0.05 *</td>
<td>2.28 ± 0.14</td>
<td>3.56 ± 0.10</td>
</tr>
<tr>
<td>2h/day exposure for 30 days</td>
<td></td>
<td>0.30 ± 0.01 *</td>
<td>2.20 ± 0.20</td>
<td>3.35 ± 0.16 *</td>
</tr>
</tbody>
</table>

* mean values significant p ≤ 0.05  Values = mean ± SE .

There was a significant (p≤0.001) increase in period of mixing males with normal females until parturition in both exposure groups as compared to control group . Also there was a significant (p≤0.001) decrease in number of pups in each parturition in both exposure groups as compared to control group , (Table 4).

Table (4) Effect of 900MHz RF-EMW from mobile phone on fertility test parameters
Groups & males with females until parturition & in each parturition & percentage %
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Control & 26 & 9 & 88
1h/day exposure for 30 days & 33*** & 6*** & 61
2h/day exposure for 30 days & 31*** & 7*** & 66

*** = mean highly significant $p \leq 0.001$, Values = mean ± SE.

The decrease in total sperm number, decrease in live sperm percentage and increase in abnormal sperms percentage which happen due to hormonal disturbance represented by decrease in serum LH, FSH levels although the decrease of serum LH level was not significant. This result was similar to other authors (Agarwal et al., 2008), (Agarwal et al., 2007), (Ozguner et al., 2005), (Yan et al., 2007). Decrease in serum testosterone level occur perhaps due to the lack of Leydig cells in seminiferous tubules cells due to the decrease in serum LH level although it is not significant which is responsible for differentiation of Leydig cells from mesenchymal cells and these Leydig cells are the main source of testosterone production and secretion, this result agree with (Ozguner et al., 2005) who found that the biological and morphological effect of mobile phone on testis causes damage of seminiferous epithelium and decrease germinal epithelium and decrease in serum testosterone level. The increase in period from mixing experimental males with normal females until parturition for fertility test, decrease in pregnancy percentage and number of pups per parturition may be due to disturbances in sperm number and its morphology and time taken for correcting the side effect that happen in sex organs tissues and this is the main cause of low fertility.

Conclusion:
The results revealed that exposure to mobile phone radiation leads to low fertility, so warranty should be taken when use this technology.

Acknowledgment:
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400 غرام، حيث قسمت إلى 3 مجاميع هي مجموعة السيطرة و مجموعة التعرض ساعة \\ يوم و مجموعة التعرض ساعتين \\ يوم \\ و لفترة 20 يوما و في نهاية التجربة قسمت المجموعات بدورها إلى مجتمعات فرعية لإجراء فحوصات السائل المنوي والبحوث الهرمونية وأجزاء تجربة الحيوية. أظهرت النتائج انخفاضا معنويا في أوران الخصى لمجامعي الخصى و أنخفاضا في وزن البروستات في مجامع التعرض مقارنة بالسيطرة و لوحظ زيادة في وزن الحويصلة المنوية في مجموعات التعرض ساعتين \\ يوم مقارنة بالسيطرة و إنخفاضا معنويا في العدد الكلي للنطف لمجامعي المعالجة مقارنة بالسيطرة و الدراسة مع أن زيادة معنوية في نسبة النطف المثبتة لمجامعي التعرض ساعتين و ساعتين \\ يوم مقارنة بالسيطرة و كذلك زيادة في النسبة المنوية للتشوهات في النطف في مجموعات التعرض مقارنة بالسيطرة ، و لوحظ من نتائج تجربة الخصوبة وجود زيادة معنوية في عدد أيام خط ذكور مجاميع السيطرة و التعرض مع إناث بالقوة موثقة لحين الولادة و انخفاض في عدد المواليد لكل ولادة في مجامع التعرض مقارنة بمجموعة السيطرة . تستنتج من ذلك أن الأمواج الكهرومغناطيسية الصادرة من الاتصالات لها تأثير ضار في خصوبة ذكور الجرذان .

References :
