Egyptian Journal of Anatomy, Jul. 2017; 40(2):265-278

Original Article

The Effect of Mobile Phone Exposure on the Testicular Structure of the Pubertal and the Adult Male Albino Rat

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ABSTRACT

Introduction: Nowadays, mobile phones (MP) have become indispensable devices in our daily life. The electromagnetic waves and biological effects of MP have recently become a crucial subject in scientific studies. As men usually put MP in standby position most of the day in their pockets close to their testes, the assessment of consequences of MP handling on the male reproductive function seems to be of great importance. Electromagnetic exposure has been found to alter the reproductive endocrine hormones and gonadal function in both the pre-pubertal and the adult rats. Other studies declared that prolonged MP use has been associated with a delay in the onset of puberty.

Aim of work: To study the histological structure of the testis of both pubertal and adult male albino rats, upon exposure to MP.

Material and Methods: Forty male albino rats were used in this study, twenty pubertal, aging 6-8 weeks and weighing 140-160 gm, and twenty adult, aging 4-6 months and weighing 200 - 250 gm. Group I (Control Group): composed of twenty rats that were left without mobile exposure and were used as control. It was further subdivided into: Group IA (Control Pubertal Group): consisted of ten pubertal rats. Group IB (Control Adult Group): consisted of ten adult rats. Group II (Mobile Group): composed of twenty rats that were exposed to MP during calling for 1 month. It was further subdivided into: Group IIA (Mobile Pubertal Group): consisted of ten pubertal rats. Group IIB (Mobile Adult Group): consisted of ten adult rats. At the end of the experiment, the testis specimens were collected and processed for light microscopic and immunohistochemical examination and morphometric studies were also done.

Results: Histological examination of the testis sections of the mobile pubertal group stained with Hx. & E showed multiple focal areas of rupture of basement membrane, decrease in the height of epithelial lining of seminiferous tubules with poor discrimination between its spermatogenic stages. Semithin sections revealed multiple areas of patchy depletion of germinal epithelium and some of the existing spermatogenic cells appeared with pyknotic nuclei. An increase in the number of Leyding cells was observed in the interstitial tissue. Immunohistochemical examination for Caspase III showed positively stained cells revealing marked apoptosis. On the other hand, examination of testis sections of the mobile adult group stained with Hx. & E. showed distortion in some seminiferous tubules. Multiple areas of degeneration were noted close to the basement membrane in many tubules. The thickness of the germinal epithelium was relatively decreased with areas of patchy depletion observed among the spermatogenic cells Moreover, some tubules showed disturbance of their normal architecture with almost complete loss of discrimination of their spermatogenic stages . Secretions were noticed in the interstitial tissue with notable adhesion of adjacent tubules. In semithin section Sertoli cells and spermatogonia were few in number .Some primary spermatocytes appeared distorted in shape. Vacuolation in between the seminiferous layers was evident, along with areas of complete depletion from cellular content. An increase in the number of Leydig cells was observed in the interstitial tissue. Immuno-histochemical examination for Caspase III showed positively stained cells revealing evident apoptosis.

Conclusion: Affection of the seminiferous epithelium following mobile waves' exposure and the more marked affection observed in the pubertal rats in our study, may be considered as a danger alarm for the widely spread and increasing use of mobile phones among pubertal teenage males. Therefore, further studies regarding this age group in human and the possible protective agents against harmful waves are highly recommended.

Key Words: Adult, Mobile, pubertal, testis

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 DOI: 10.21608/EJANA.2018.16905

INTRODUCTION

Nowadays, mobile phones (MP) have become indispenable devices in our daily lives. There is an increasing use of digital MP, using protocols as Global System for Mobile Communication (GSM). These MP operate on wireless technology with communication occurring via 900 – 1800 MHz signal that is pulsed at 217 Hz (*Barutcu et al*., *2011*). The electromagnetic waves (EMW) and biological effects of MP have recently become a decisive subject in scientific studies (*Dasdag et al*., *2009*).

Some studies revealed that EMW increase the oxidative stress and apoptosis in some tissue cells (*Dasdag et al*., *2009*). Moreover, *Akif et al*., (*2013*) stated that the use of MP increased oxidative stress and apoptosis in both calling and standby positions. However, the subsequent expected ameliorating effect of anti-oxidants could not be reported in either oxidative stress or apoptosis.

As men usually put MP in standby position in their pockets, close to the testes most of the day, assessment of consequences of MP handling on male reproductive function seems to be of great importance (*Agarwal et al*., 2008). *Gorpinchenko et al*., (*2014*) recorded that there is a correlation between MP radiation exposure, DNA–fragmentation level and decreased sperm motility. Therefore, recent concerns over long-term exposure to the EMW emitted by MP should be taken more seriously, keeping in mind the notable relative deterioration of the male germ cell line including spermatogenesis and sperm maturation (*Aitken et al*., *2004*). Recent studies were done on the effect of MP on the male reproductive function both in humans (*Agarwal et al*., *2008*) and animals (*Yan et al*., *2007*). However, results of these studies were conflicting, as some revealed negative findings (*Riberio et al*., *2007*), whereas others indicated that EMW have a wide spectrum of detrimental effects on sperm parameters (*Yan et al*., *2007*). Regarding endocrinal aspects, EMW exposure was found to alter the reproductive endocrine hormones and the gonadal function in both pre-pubertal and adult rats (*Myung* and *Chan*, *2012*). In addition, *Burch et al*., (*2002*) declared that prolonged MP use (>25 min/day for 2 weeks) has been associated with delay in the timing of the onset of puberty. However, *Salama et al*., (*2010*) stated that the cumulative effects of exposure to EMW emitted by a conventional MP in standby position on the testicular structure and function were not yet fully investigated.

Hence, it became the aim of the present work to study the possible changes in the histological structure of the testis of both pubertal and adult male albino rats, upon exposure to MP.

MATERIAL AND METHODS

Animals:

Forty male albino rats were obtained from the Animal House of The Bilharzial Research Unit, Ain Shams University. The animals were housed in conventional wire-mesh cages in a room temperature regulated at 21 ±10˚C, humidity 45-50%, and light/dark cycles (/12h). The rats were fed on standard rat diet and allowed free water access. Animals were allowed to acclimatize to experimental conditions by housing them for 10 days prior to experiment.

Exposure System: Rats were taken to the exposure room and then exposed to the EMR emitted from a commercially available GSM cellular phone (900-1800 MHz) during calling (it was only rung, not switched on) for 10 minutes every 1 hour for 8 hours each day and were left in standby mode at the remaining times of the day over the course of 1 month (*Akif et al., 2013*). The control animals were exposed to a mobile phone without a battery in similar cages for the same period in a separate but similar room to standardize the same environmental conditions.

Experimental protocol:

Animals were divided into two groups, each containing twenty rats, ten pubertal and ten adult. Pubertal rats aged rats 6-8 weeks and weighed 140-160 gm and adult rats aged 4-6 months and weighed 200-250 gm.

- **Group I (Control Group):** consisted of twenty rats that were left without mobile exposure and were used as control.

It was further subdivided into:

- **Group IA (Control Pubertal Group):** contained ten pubertal rats.
- **Group IB (Control Adult Group):** contained ten adult rats.

 Group II (Mobile Group): consisted of twenty rats that were exposed to mobile phone during calling for 1 month.

It was further subdivided into:

- **Group IIA (Mobile Pubertal Group):** contained ten pubertal rats.
- **Group IIB (Mobile Adult Group):** contained ten adult rats.

At the end of the experiment, all rats were anaesthetized using ether inhalation. The testes were dissected and processed for both paraffin and semithin sections to be examined by the light microscope.

Specimens for light microscopic studies were fixed in 10% formalin in water for one week. After fixation, tissues were dehydrated in ascending grades of ethanol, cleared in xylol and embedded in paraffin blocks. Sections of 5 µm in thickness were cut and stained with Haematoxylin and Eosin Stain. (*Bancroft and Gamble, 2008*)

Specimens for the semithin sections (Toluidine blue) were immediately cut into cubes (1mm in diameter) and fixed overnight in 2.5% phosphate-buffered glutaraldehyde (pH 7.3) at 4°C. Postfixation in 1% buffered osmium tetroxide for 1–2 h was followed by dehydration in ascending grades of ethyl alcohol, cleared in propylene oxide, and finally embeded in fresh Epon capsules. Semithin sections 1 µm in thickness were cut with a glass knife and stained with toluidine blue and then examined by an Olympus light microscope (Bancroft & Gamble, 2008).

To demonstrate any possible apoptosis, immunohistochemical staining for Caspase III was performed on 5 micron thick paraffin sections. Testes sections were deparaffinized and hydrated in 3% H2O2 for 5 min and rinsed with PBS for 15 min. The sections were blocked with 1.5% normal goat serum in phosphate buffered saline (PBS) and then incubated (45 min, room temperature) with rabbit polyclonal antihuman caspase-3 (0.5µg/ml) in 1.5% normal goat serum in PBS. The sections then were incubated with biotin-conjugated goat anti-rabbit IgG (1:200, 1 h, room temperature), avidin-biotin-peroxidase complex (Santa Cruz Biotechnology, Inc., rabbit peroxidase kit; 1 h) and DAB solution. Sections were counterstained with hematoxylin. For negative controls, rabbit IgG (1 µg/ml) instead of the primary antibody was added to the reaction (*Kim et al., 2011*).

Morphometric Study:

Morphometric analysis was carried out on routine Haematoxylin and Eosin stained slides using Leica *Qwin 500* Image Analyzer computer system in Image Analyzer Unit, Military Medical Academy. The height of germinal epithelium and the surface areas of lumen of tubules were measured. The measurements were repeated on several serial sections. The image analyzer was first calibrated automatically to convert the measurement units (pixels), produced by the image analyzer program into actual micrometer units.

Statistical analysis:

The data were analyzed using MedCalc® Version 11.1.1.0 for Windows (MedCalc Software, Belgium) and Microsoft Office Excel 2010 (Microsoft, USA). Mean, standard deviation and Student T test were done to compare both the pubertal and adult mobile groups with their control ones. P values were obtained and interpreted as follows; $p>0.05$ were considered statistically insignificant, $p<0.05$ were considered statistically significant and $p<0.001$ were considered to be highly significant.

RESULTS

Group I (Control Group):

Group IA: (Control Pubertal Group)

Histological examination of testis sections of the control pubertal group stained with Hx. & E revealed that the seminiferous tubules were rounded to oval (Fig.1), separated by minimal amount of interstitial tissue (Figs.1&2) containing Leydig cells (Fig.2) and lined by almost mature germinal epithelium (Fig.2). This germinal epithelium showed pyramidal Sertoli cells and oval spermatogonia with darkly stained nuclei close to the basement membrane (Fig.2). Moreover, some of these tubules showed eosinophilic threads in their lumina representing tails of the sperms (Figs. 1&2).

Semithin sections showed seminiferous tubules enveloped by a layer of spindle shaped myoid cells on the outer surface of the basement membrane (Figs.3&4). Pale stained Sertoli cells and rounded spermatogonia with heterochromatic nuclei were seen resting on the basement membrane (Fig.3).The primary spermatocytes, with their characteristic patchy chromatin condensed nuclei, were the largest cells seen in the tubule (Fig.3). Inner to the spermatocytes, rounded early spermatids with central rounded nuclei were seen arranged in two or three layers (Fig.4). In addition, few elongated spermatids with deeply stained nuclei were observed (Fig.4). Leydig cells and blood vessels were noticed in the interstitial tissue among the tubules (Fig.4).

Immunostained sections showed negative staining to caspase III (Fig.5).

Group IB: (Control Adult Group)

Histological examination of testis sections of the control adult group stained with Hx.&E. showed rounded to oval seminiferous tubules, arranged almost closer to each other than those of the pubertal group (Fig.6). The tubules were lined by multiple layers of germinal epithelium and separated by interstitial tissue containing blood vessels and Leydig cells (Figs. 7&8). The lumen of most of the tubules was filled with esinophilic threads representing tails of the sperms (Figs 6,7&8) with a characteristic whorly appearance observed in some tubules (Fig.7). Pyramidal Sertoli cells and oval spermatogonia with dark stained nuclei were observed close to the basement membrane (Fig.8).

Semithin sections examination showed spindle shaped myoid cells resting on the outer surface of the basement membrane, pale stained pyramidal Sertoli cells and more darkly stained rounded spermatogonia with heterochromatic nuclei were observed. Large primary spermatocytes with their patchy chromatin were seen (Figs 9&10). Two or three layers of rounded spermatids with acrosomal cap together with elongated spermatids were frequently observed. Mature spermatids were noticed anchoring the lumen of many tubules (Fig.9). The interstitial tissue showed apparent increase in Leydig cells (Fig.10).

Immunostained sections showed negative staining to caspase III (Fig. 11).

Group II (Mobile Group):

Group IIA: (Mobile Pubertal Group)

Histological examination of testis sections of the mobile pubertal group stained with H & E showed some seminiferous tubules with focal areas of disruption of basement membrane (Figs12&13) while others had almost complete absence of their basement membrane lining and appeared coalesed with neighboring ones (Fig.14). Detachment of seminiferous epithelium from the basement membrane was evident in many tubules (Figs 12&15) with depletion of many of the basal cells (Figs.14&15). In addition, apparent thinning of germinal epithelium was observed together with focal areas of depletion from its cellular content (Fig.15). Poor discrimination of the spermatogenic stages was also encountered (Figs.14&15). Moreover, the lumina of some tubules were seen to be obsecured by shedded dark nuclei (Fig.13).

Semithin sections showed marked disturbance in the testicular architecture with notable decrease in the basal Sertoli cells and spermatogonial layer. Marked degenerative changes in the form of; multiple areas of patchy depletion of germinal epithelium (Figs.16&17) and pyknosis of the nuclei of the existing spermatogenic cells (Fig.16) were encountered. In addition, some spermatids lost their adluminal position and were seen intermingled among the spermatogenic cell layers (Fig.16). Apparent increase number of Leydig cells was also observed in the interstitial tissue (Fig. 17).

Immunostained sections showed strong positive staining for Caspase III more evident in the parabasal cells and revealing marked apoptosis (Figs 18).

Group IIB: (Mobile Adult Group)

Histological examination of testis sections of the mobile adult group stained with Hx. & E. showed distortion of architecture in some seminiferous tubules (Figs.19&20) with focal areas of basement membrane disruption (Fig.20). Areas of detachment of germinal epithelium from the basement membrane with focal depletion of basal cells were also evident in some tubules (Figs.19&21). Some Sertoli cell nuclei were seen in the adluminal compartment separated from the

basement membrane (Fig.21). The thickness of the germinal epithelium was relatively decreased (Fig.19) and some tubules showed almost complete loss of discrimination among their spermatogenic stages (Fig.20). The lumen of the tubules was filled with scanty eosinophilic threads representing few sperm tails (figs.19&20) with almost complete loss of the characteristic whorly appearance noticed in group IIB. Moreover, some tubules appeared to have almost empty lumina (Fig.19). Exudation was also noticed in the interstitial tissue with notable coalesion of adjacent tubules (Fig.20).

Semithin section clarified the disturbance in the testicular architecture. Sertoli cells and spermatogonia were relatively fewer compared to group IB (Fig.22). Many degenerative changes were encountered in the germinal epithelium including; vacuolation (Fig.22), areas of focal cellular depletion (Fig.23) and obvious primary spermatocytes diminution with karyolysis detected in some of them (Fig.23). Rounded spermatids were also fewer as compared to the control adult group (Fig.22) and were seen nearer to the basement membrane with loss of acrosomal cap (Fig.23). Moreover, groups of spermatids were seen adherent to eachother without cellular demarcation (Fig.23). Leydig cells were observed in the interstitial tissue (Fig.22).

Immunostained sections showed positive staining of many parabasal cells for Caspase III revealing evident apoptosis (Fig.24).

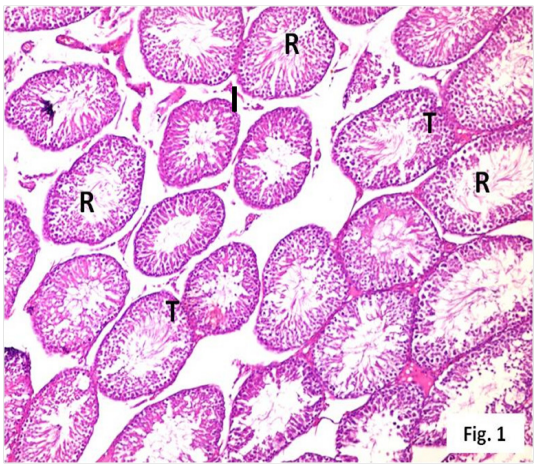

Fig. 1: A photomicrograph of a section of the testis of a control pubertal (group IA) albino rat showing rounded to oval seminiferous tubules (T) separated by minimal amount of interstitial tissue (I). Note the eosinophilic threads representing sperm tails (R) inside some of them. Hx.&E.; X100.

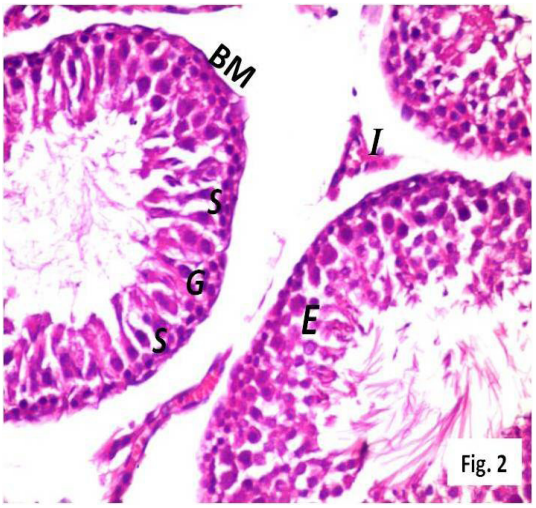

Fig. 2: A photomicrograph of a section of the testis of a control pubertal (group IA) albino rat showing seminiferous tubules lined by layers of almost mature germinal epithelium (E) with some eosinophilic threads in their lumina representing sperm tails (R). Note the pyramidal Sertoli cells (S) and oval spermatogonia with dark nuclei (G) close to the basement membrane (BM). Note also the minimal amount of interstitial tissue (I) among tubules. Hx.&E.; X400.

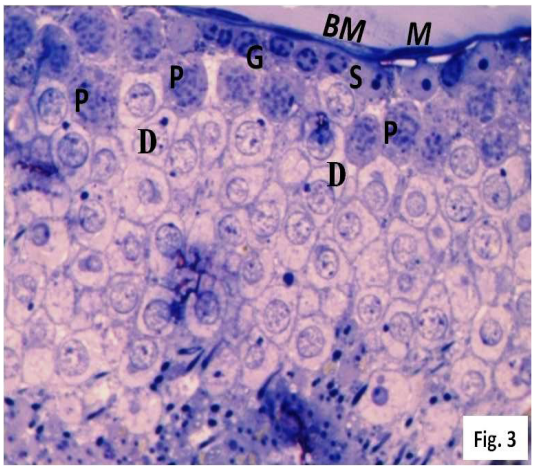

Fig. 3: A semithin section of the testis of a control pubertal (group IA) albino rat showing pale stained Sertoli cells (S) and rounded spermatogonia with heterochromatic nuclei (G) close to basement membrane (BM). Note the large primary spermatocytes with coarse chromatin condensed in the nuclei (P) and the rounded spermatids (D). Note also the spindle shaped myoid cells (M) in the tubular linning. Toluidine blue; X1000.

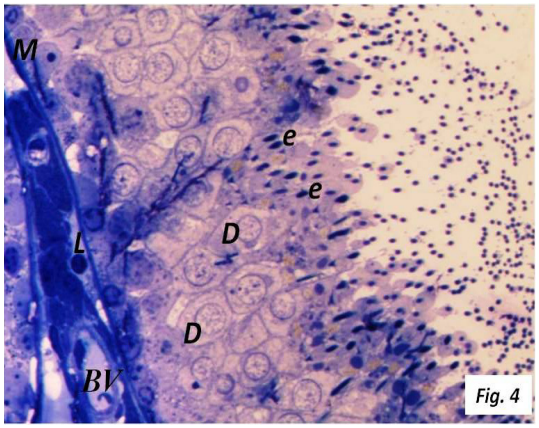

Fig. 4: A semithin section of the testis of a control pubertal (group IA) albino rat showing rounded early spermatids with central rounded vesicular nuclei (D) together with some elongated spermatids (e). Note the spindle shaped myoid cells (M) in the tubular linning and the Leydig cell (L) and blood vessel (BV) in the interstitial tissue. Toluidine blue; X1000.

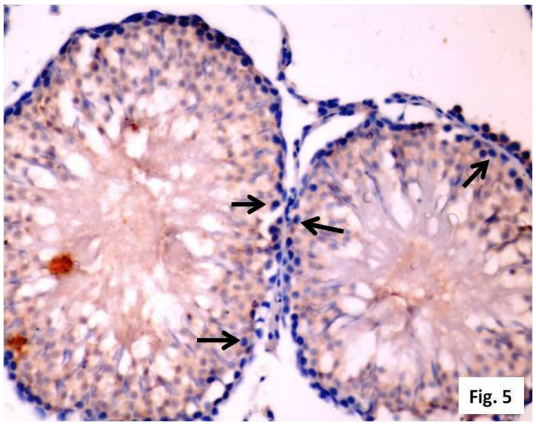

Fig. 5: A Caspase III immunohistochemical section of the testis of control pubertal (group IA) albino rats showing negatively reacting bluish cells (↑). Caspase III; X400.

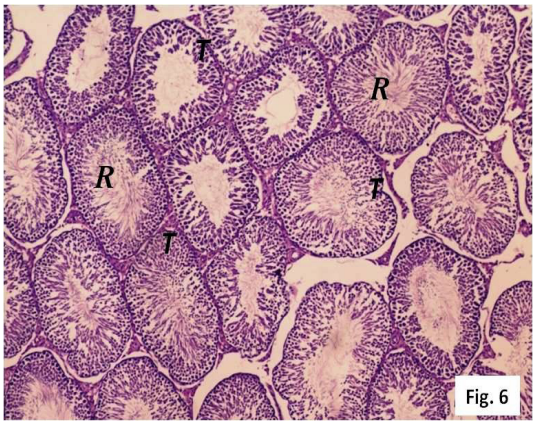

Fig. 6: A photomicrograph of a section of the testis of a control adult (group IB) albino rat showing rounded to oval seminiferous tubules (T) arranged almost closer to each other than in the pubertal group. Note the eosinophilic threads representing sperm tails (R) inside most of them. Hx.&E.; X100.

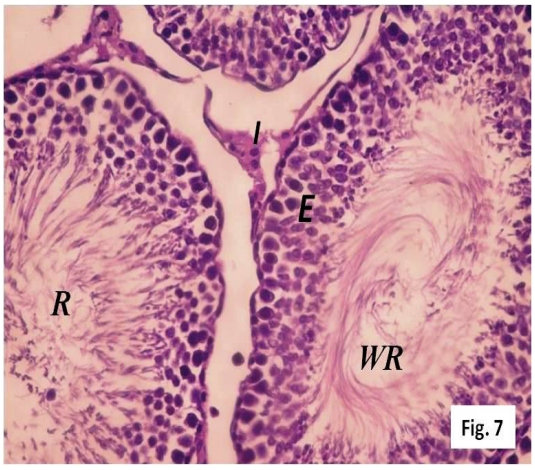

Fig. 7: A photomicrograph of a section of the testis of a control adult (group IB) albino rat showing seminiferous tubules lined by multiple layers of germinal epithelium (E). The lumen of tubules contain esinophilic threads representing sperm tails (R) and having the characteristic whorly appearance (WR) in one of them. Note the interstitial tissue (I) in between the tubules. Hx.&E.; X 400.

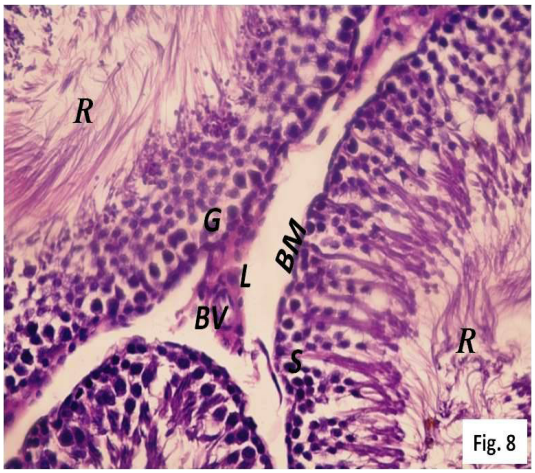

Fig. 8: A photomicrograph of a section of the testis of a control adult (group IB) albino rat showing seminiferous tubules lined by germinal epithelium containing pyramidal Sertoli cells (S) and spermatogonia with dark nuclei (G) located close to the basement membrane (BM). Note the esinophilic threads representing the sperms tails (R) in the lumen of tubules and the blood vessel (BV) and Leydig cells (L) in the interstitial tissue. Hx.&E.; X400.

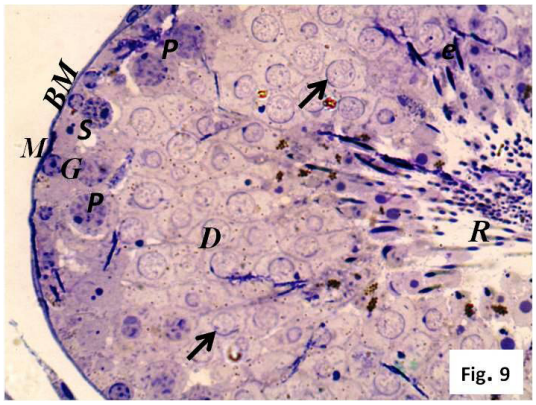

Fig. 9: A semithin section of the testis of a control adult (group IB) albino rat showing pale stained Sertoli cells (S) and spermatogonia with heterochromatic nuclei (G) close to basement membrane (BM). Note the large primary spermatocytes with patchy chromatin (P), the multiple layers of rounded spermatids (D) with acrosomal cap (↑) and the elongated spermatids (e). Note also the mature spermatids (R) in the lumen. And the spindle shaped myoid cell (M) in the tubular lining. Toluidine blue; X1000.

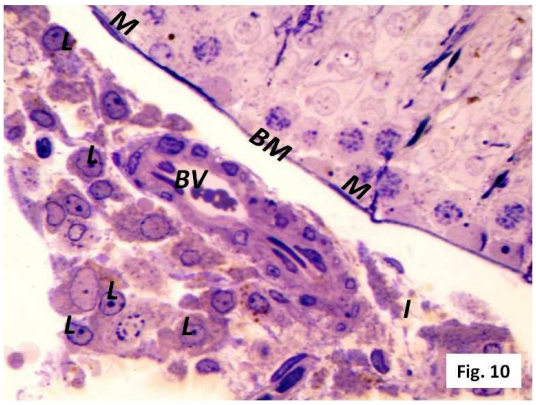

Fig. 10: A semithin section of the testis of a control adult (group IB) albino rat showing seminiferous tubule with spindle shaped myoid cells (M) close to the basement membrane (BM). Note the blood vessel (BV) and the apparent increase of Leydig cells (L) is noticed in the interstitial tissue (I). Toluidine blue; X1000.

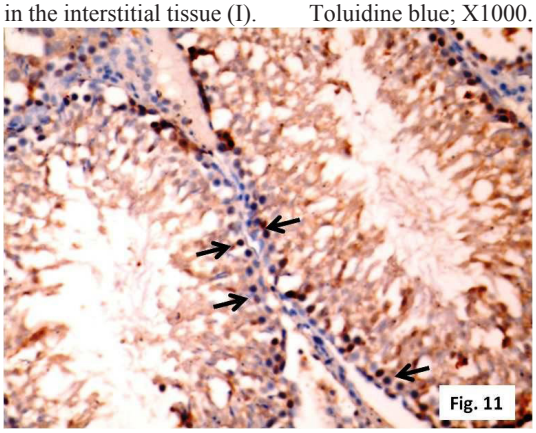

Fig. 11: A Caspase III immunohistochemical section of the testis of control adult (group IB) albino rats showing negatively reacting bluish cells (↑). Caspase III; X 400.

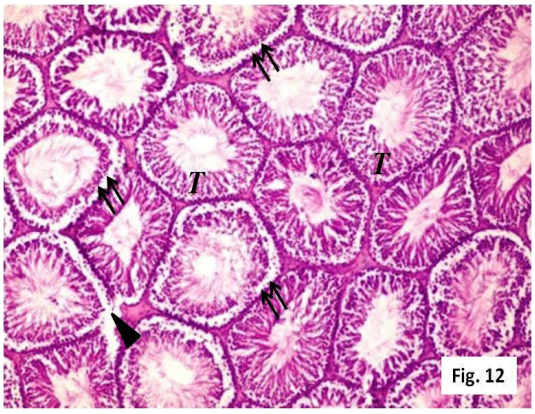

Fig. 12: A photomicrograph of a section of the testis of a mobile pubertal (group IIA) albino rat showing seminiferous tubules (T) whose germinal epithelial cells were detached from the basement membrane (↑↑). Note the area of focal basement membrane disruption (▲) in one of the tubules. Hx.&E.; X100.

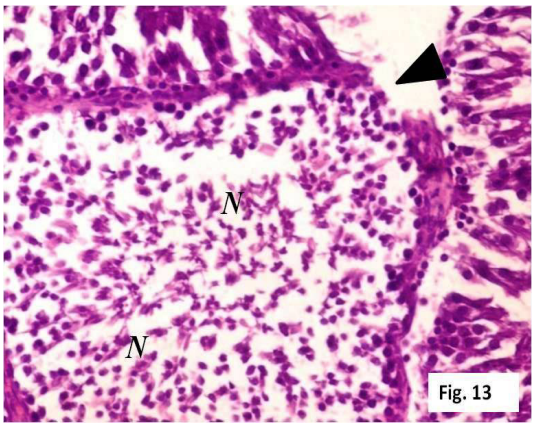

Fig. 13: A photomicrograph of a section of the testis of a mobile pubertal (group IIA) albino rat showing of seminiferous tubule with striking loss of the normal architecture of germinal epithelium, studding of the lumen by shedded dark nuclei (N) and focal rupture of its basement membrane (▲). Hx.&E.; X400.

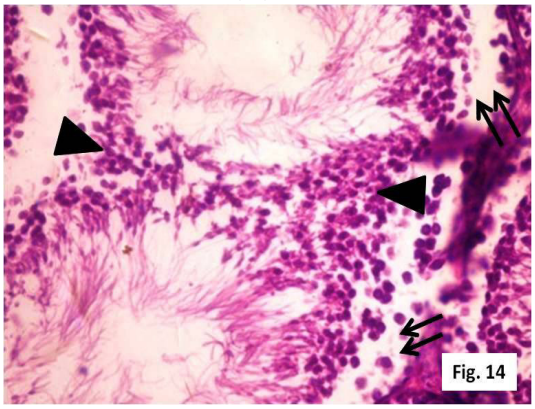

Fig. 14: A photomicrograph of a section of the testis of a mobile pubertal (group IIA) albino rat showing seminiferous tubules with almost complete absence of their basement membrane leading to their coalescence (▲). Note the focal depletion of basal cells (↑↑) and the poor discrimination between the spermatogenic stages. Hx.&E.; X400.

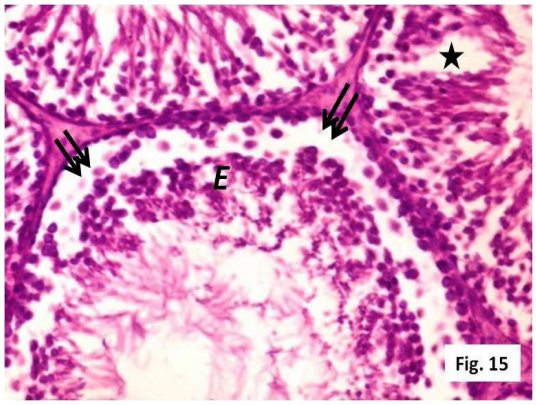

Fig. 15: A photomicrograph of a section of the testis of a mobile pubertal (group IIA) albino rat showing detachement of the seminiferous cells from the basement membrane with focal depletion of basal cells (↑↑), apparent thinning of the germinal epithelium (E) with poor discrimination of the remaining spermatogenic cells. Note the area of focal cellular depletion (*). Hx.&E.; X400.

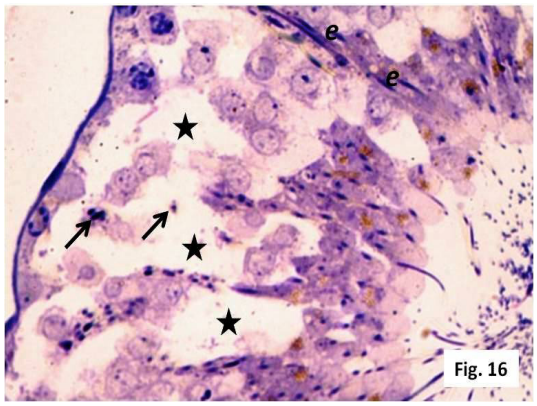

Fig. 16: A semithin section of the testis of a mobile pubertal (group IIA) albino rat showing disturbance in architecture, decrease in Sertoli and spermatogonial cells, together with areas of patchy depletion (*) of germinal epithelium. Spermatids (e) are seen intermingled among the different spermatogenic layers. Note the piknotic nuclei (↑) of some spermatogenic cells. Toluidine blue; X1000.

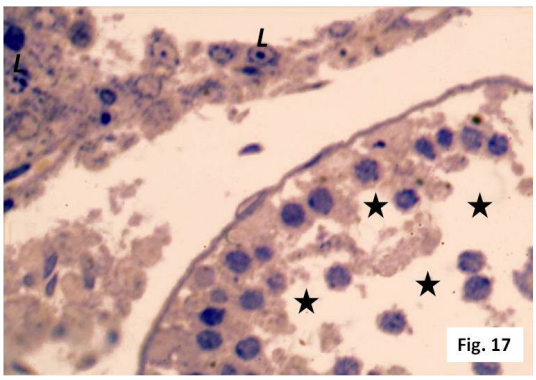

Fig. 17: A semithin section of the testis of a mobile pubertal (group IIA) albino rat showing areas of patchy depletion (*) of germinal epithelium. Note the Leydig cells (L) in the interstitial tissue (I). Toluidine blue; X1000.

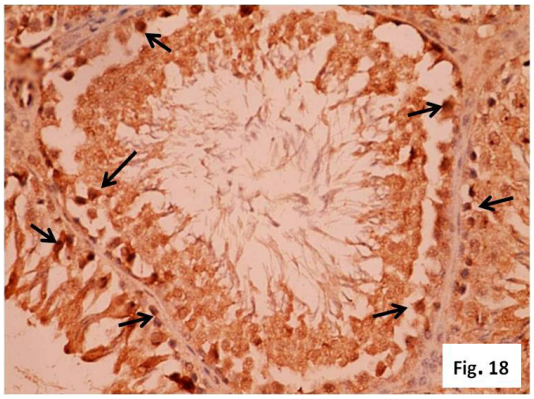

Fig. 18: A Caspase III immunohistochemical section of the testis of a mobile pubertal (group IIA) albino rat showing positively stained dark brown cells (↑) revealing marked apoptosis. Caspase III; X400.

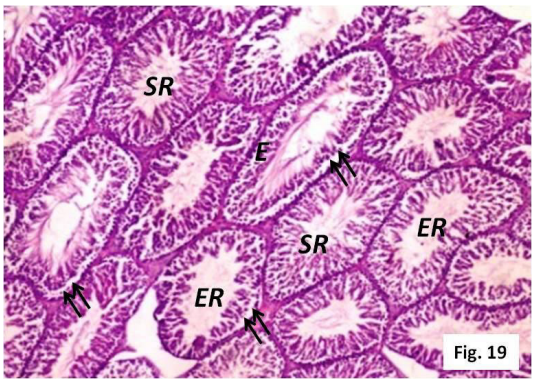

Fig. 19: A photomicrograph of a section of the testis of a mobile adult (group IIB) albino rat showing seminiferous tubules with some distortion in architecture, relative decrease in germinal epithelial thickness (E) and multiple areas of germinal epithelial cells detached from the basement membrane (↑↑). Note, that the luminae of some tubules contain scanty eosinophilic threads representing few sperm tails (SR) while that of others appear almost empty (ER).

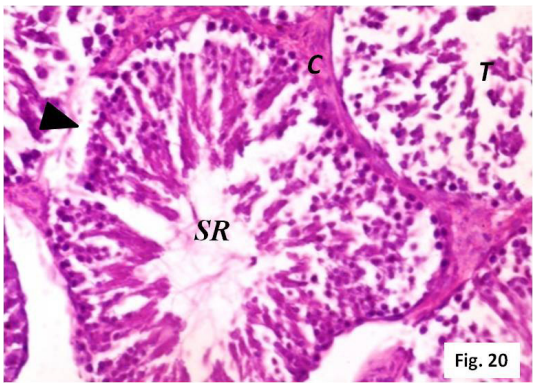

Fig. 20: A photomicrograph of a section of the testis of a mobile adult (group IIB) albino rat showing a distorted seminiferous tubule with focal rupture of its basement membrane (▲), almost complete loss of discrimination of its spermatogenic cells and scanty eosinophilic threads representing few sperm tails (SR) in its lumen. Note the evident loss of architecture in the adjacent tubule (T) and the exudation (C) between tubules. Hx.&E.; X400.

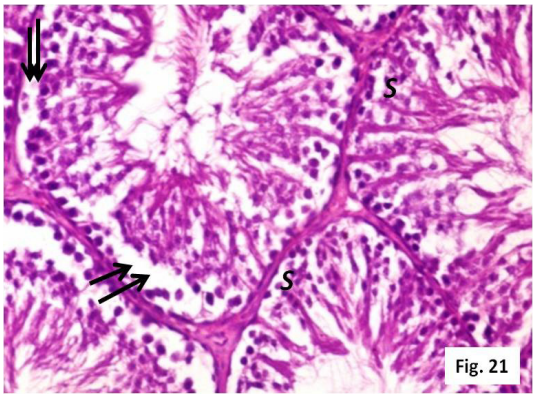

Fig. 21: A photomicrograph of a section of the testis of a mobile adult (group IIB) albino rat showing areas of focal detachment of the spermatogenic cells from the basement membrane with focal depletion of basal cells (↑↑). Note the Sertoli cell nuclei seen in adluminal compartment (S) and separated from the basement membrane. Hx.&E.; X400.

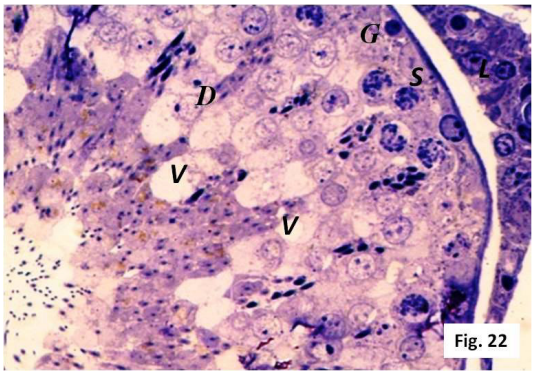

Fig. 22: A semithin section of the testis of a mobile adult (group IIB) albino rat showing disturbance in testicular architecture with vacuolation (V) in the germinal epithelium.. Note the relatively fewer Sertoli cell (S), spermatogonia (G) and rounded spermatids (D) compared to those observed in the control adult group. Note also the leydig cells (L) in the interstitial tissue. Toluidine blue; X1000.

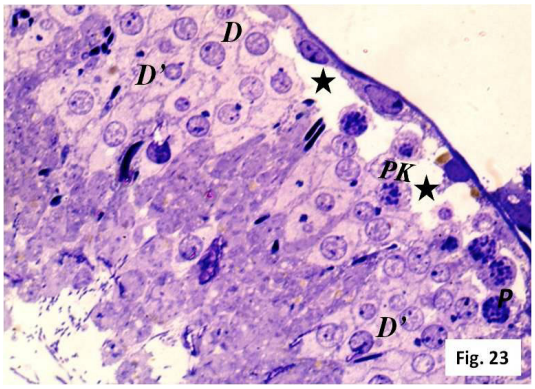

Fig. 23: A semithin section of the testis of a mobile adult (group IIB) albino rat showing areas of cellular depletion (*) and obvious primary spermatocytes (P) diminution with karyolysis detected in one of them (PK). The round spermatids (D) were seen nearer to the basement membrane with loss of acrosomal cap and some of them were adherent without cellular demarcation (D'). Toluidine blue; X1000.

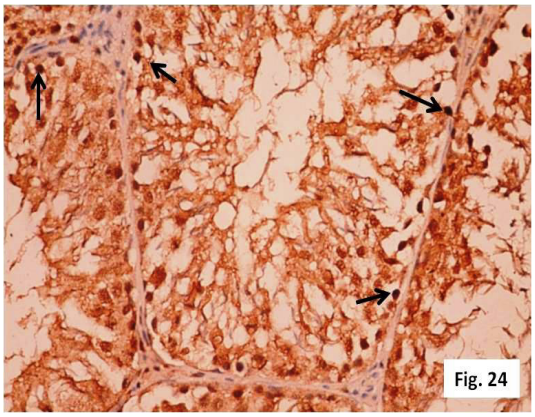

Fig. 24: A Caspase III immunȩhistochemical section of the testis of a mobile adult (group IIB) albino rat showing positively .stained brownish cells (↑) revealing apoptosis. Caspase III; X 400.

Table 1: shows the results of the micrometric parameters of the testis.

	Area of the lumina of seminiferous tubules (μm²)	Height of germinal epithelium (μm)
Control Pubertal (group IA)	1379.48 ±. 172.42	11.99 ± 2.47
Control Adult (group IB)	2180.89 ±. 343.76	21.85 ± 5.81
Mobile Pubertal (group IIA)	4898.59 ±. 983.75	8.75 ± 2.06
Mobile Adult (group IIB)	3030.23 ± 343.76	20.11 ± 2.46

A) The mobile pubertal group showed statistically highly significant increase ($p < 0.001$) in the area of the lumina of seminiferous tubules and significant decrease ($p < 0.05$) in the height of germinal epithelium compared to its control group.

B) The mobile adult group showed statistically significant increase ($p < 0.05$) in the area of the lumina of seminiferous tubules but the height of the germinal epithelium showed an insignificant decrease ($p > 0.05$) compared to its control group.

DISCUSSION

The Mobile phones have become a vital part of everyday life. These cellular devices emit radio frequency EMW that were observed to be associated with an obvious decrease in male fertility (*Bhat, 2013*). These devices are nowadays vastly spread among both adults as well as teenage males.

The present work revealed significant changes in the histological structure of the testes of both adult and pubertal rats exposed to mobile waves. These changes include; distortion of seminiferous tubules and thinning of germinal epithelium which were more marked in the pubertal mobile group than the adult one compared with their corresponding control groups. Also, focal areas of basement membrane rupture were observed in the two mobile groups but the picture was aggravated in the mobile pubertal group, where some tubules showed almost complete absence of basement membrane with subsequent coalescence of tubules. Similarly, *Al Damegh (2012)* noticed degenerative changes in seminiferous tubules with complete absence of spermatozoa and significant decrease in the mean height of germinal epithelium in adult rats exposed to EMW emitted from MP. The author stated that these waves had a negative effect on testicular function through the induction of oxidative stress with concomitant disruption of the testicular antioxidant status. This was previously observed by *Ozguner et al., (2006)* who stated that the EMW produced by MP increase the oxidative stress on various tissues. Moreover, the power of vitamins C and E as antioxidants, given in conjunction with the EMR exposure, to ameliorate this pathology has been found to facilitate the restoration of testicular tissue morphology and function thus confirming the oxidative pathology and their importance to overcome it *(Al Damegh 2012)*.

The present study observed that most of spermatids in the mobile adult group showed absence of acrosomal cap that was noticed in the control adult group. In addition, some spermatids in mobile pubertal group lost their adluminal position and were seen intermingled among cells of previous series. *Kesari et al., (2011)* declared that radiofrequency electromagnetic waves from commercially available cell phones may affect the fertilizing potential of spermatozoa. *Agrawal et al., (2008)* added that the use of cell phones decrease the semen quality in men by decreasing the sperm count, motility, viability and normal morphology.

Our study also showed many tubules with poor discrimination among germinal epithelial cells and a significant decrease in the germinal epithelial height following mobile wave exposure and this was more marked in the pubertal group. Among the few researches done on the pubertal rats in this context, *Kim et al., (2007)* studied the adverse effects of electro-magnetic waves on the proliferation and differentiation of spermatogonia in pubertal age and observed degeneration of the germinal epithelium and decrease in spermatogenic count. In agreement with the present study, *Aydin et al. (2007)* found that exposure of rats to EMF caused deceleration of spermatogenesis and degeneration of germ cells. The exposure of mice to elecrtromagnetic field for 12 days also induced an increase in maturation arrest in spermatogenesis, disorder in germinal cell distribution and a decrease in germ cell population *(Khayyat, 2011)*.

In the present study, immunohistochemical examination for Caspase III showed moderate apoptosis in adult rats and more intense apoptosis in pubertal rats, *Akif et al., (2013)* declared that significant increase in apoptosis was observed in testis of adult rats subjected to electromagnetic waves emitted from mobile phones and attributed these negative effects to the induction of oxidative stress impacted on the testicular tissue. This could also explain the patchy depletion encountered in our study throughout the germinal epithelium in the two mobile groups. This degenerative finding was again more marked in the pubertal group. Pyknotic nuclei were encountered among spermatogenic cells and exfoliated dark nuclei were noticed in the lumina of tubules. *Khaki et al., (2008)*, stated that mobile waves have the abilitly to generate destructive reactive oxygen species including superoxide, hydrogen peroxide and hydroxyl radical and frequently lead to produce oxidative and necrotic damages. On the contrary, *Dasdag et al (2008)* investigated the effects of 900 MHz RF waves emitted by a GSM stimulator on spermatogonia in rat seminiferous tubules. No statistically significant difference was found between the groups and RF waves did not cause apoptosis during spermatogenesis.

Regarding Sertoli cells, immunohistochemical studies showed positive staining of parabasal cells including Sertoli cells. Haematoxylin & Eosin stained sections revealed that in adult rats exposed to mobile waves, these cells lost their basal position and acquired an adluminal one. *Mackay et al., (1999)* found that the structure and function of Sertoli cells are dependent on the Sertoli cell - basement membrane interactions. *Dirami et al, (1995)* also stated that detachment of Sertoli cells from the matrix and absence of basement membrane resulted in apoptosis. Moreover, *Meehan et al, (2000)* mentioned that many factors essential for germ cell development are synthesized by Sertoli cells and that any agent that impairs the viability and function of these cells may lead to profound effects on spermatogenesis. This could additionally explain the poorly discriminated spermatogenic cells, few spermatogonia, distorted primary spermatocytes and the almost empty lumina noticed also in this group.

Lastly, most of the studies that examined the effect of mobile waves on rat's testis were done on the adult rats and few were done on the pubertal rats. However, our study aimed to compare and contrast the effects of mobile waves on the pubertal versus the adult rats. We observed that both age groups were greatly affected but with more marked and destructive effects on the rats in the pubertal age group.

CONCLUSION

Testicular structure affection following mobile waves' exposure and the more marked affection of the pubertal rats observed in our study, may be considered as a danger alarm for the widely spread and increasing use of MP among pubertal teenage males. Therefore, further studies regarding this age group in human and the possible protective agents are highly recommended.

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تأثير التعرض للهاتف المحمول على بنية الخصية في الجرذ الأبيض الذكر البالغ و في مرحلة البلوغ

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ملخص البحث

مقدمة : أصبحت الهواتف المحموله أجهزة لا غنى عنها حاليا في حياتنا اليومية. ولذلك احتلت الابحاث المعنيه بالموجات الكهرومغناطيسية والتأثيرات البيولوجية للهواتف المحموله اهتمام الدراسات العلمية مؤخرا. و من المعروف ان الرجال عادة ما يضعوا الهواتف المحموله في جيوبهم على مقربة من الخصية فى اغلب الاوقات على مدار اليوم. لذا فقد اصبح تقييم النتائج المترتبة من التعرض للهاتف المحمول على وظيفة الاعضاء التناسلية للذكور ذو أهمية كبرى. و قد أوضحت بعض الدراسات أن التعرض للموجات الكهرومغناطيسيه يؤدي الي تغيير في هرمونات الغدد الصماء المعنيه بالانجاب ووظيفة الغدد التناسلية في الجرذان البيضاء البالغة و ايضا التى فى مرحلة البلوغ. كما أعلنت دراسة أخرى أن استخدام الهاتف المحمول لفترات طويلة يقترن بتأخر فى سن البلوغ.

وكان الهدف من العمل : هو دراسة التركيب النسيجي للخصية في ذكور الجرذان البيضاء في فترة البلوغ وفي الجرذان البالغه بعد التعرض للهاتف المحمول.

المواد والطرق المستخدمه : استخدم فى هذه الدراسه أربعون من ذكور الجرذان البيضاء.

تم تقسيم الحيوانات إلى مجموعتين، كل منها يحتوي على عشرين من ذكور الجرذان، عشرة في مرحلة البلوغ (السن:6-8 أسابيع و الوزن: 140-160 جم) وعشرة بالغين (السن: 4-6 أشهر و الوزن: 200-250 جم.) المجموعة الأولى (مجموعة ضابطه): تتألف من عشرين من الجرذان التي تركت دون التعرض للمحمول وكانت تستخدم للمراقبة. وانقسمت هذه المجموعه الي المجموعة IA : ضمت عشرة من الجرذان في مرحلة البلوغ و المجموعة IB: ضمت عشرة من الجرذان البالغين.

اما المجموعة الثانية (مجموعة الهاتف المحمول): فقد تألفت من عشرين من الجرذان التي تعرضت للهاتف المحمول لمدة شهر. وانقسمت هذه المجموعه الي: المجموعة IIA (مجموعة الهاتف المحمول فى البلوغ): و ضمت عشرة من الجرذان في مرحلة البلوغ . و المجموعة IIB (مجموعة الهاتف المحمول فى البالغين): و ضمت عشرة من الجرذان البالغين.

في نهاية التجربة، تم جمع عينات الخصية ومعالجتها للفحص بالمجهر الضوئي والفحص المناعى.

النتائج : وأظهرتخصي المجموعة IIA زيادة واضحة في قطر الأنابيب المنوية، مقارنة مع المجموعة IA الضابطه ، كما أظهرت بعض الأنابيب أماكن متعددة من تمزق الغشاء القاعدي. وقد لوحظ انخفاض واضح في عدد طبقات الغشاء الطلائي النامي مع عدم وضوح التمييز بين مراحلها المنوية. وأظهرت مقاطع Semithin مناطق من استنزاف غير مكتمل في الغشاء الطلائي النامي وبعض الخلايا المنوية الموجودة ظهر بها نوي متغلظ وقد لوحظ زيادة عدد الخلايا Leydig في النسيج الخلالي. بينما أظهرفحص المناعية النسيجية لكاسباس الثالث تغيير اللون الملحوظ فى الكثير من الخلايا الي البني المكثف مما يدل علي موت الخلايا المبرمج.

ومن ناحية أخرى قد أظهر فحص الخصي فى المجموعة IIB تشويه في بعض الأنابيب المنوية مع زيادة واضحة في قطرالانابيب مقارنة بالمجموعة الضابطة (مجموعة(IB جنبا إلى جنب مع مناطق متعددة من الانحطاط بالقرب من الغشاء القاعدي في العديد من الأنابيب وقد

انخفض نسبيا سمك الغشاء الطلائي النامي مع مناطق استنزاف غير مكتمل بين الخلايا المنوية. علاوة على ذلك، فقد أظهرت بعض الأنابيب فقدان شبه كامل للتمييز بين مراحلها المنوية. كما لوحظت إفرازات في النسيج الخلالي مع التصاق ملحوظ بين الأنابيب المجاورة. وأظهرت مقاطعsemithin قلة عدد الخلايا المنويه.كما بدت بعض الخلايا المنوية الأولية مشوهة مع فقدان لمعظم الحيوانات المنويه . و قد لوحظ وجود فجوات في الغشاء الطلائي النامي جنبا إلى جنب مع مناطق استنزاف كامل. كما أظهر فحص المناعية النسيجية لكاسباس الثالث اللون البني فى كثير من الخلايا مما يدل علي الموت المبرمج للخلايا.

الخلاصه : اثبتت هذه الدراسة ان التعرض لموجات الهاتف المحمول تؤدى الى تغييرات جمه في هيكل الخصيه فى الجرذان البيضاء و ان هذه التغيرات وضحت وضوحا جليًا في الجرذان التي تمر بمرحلة البلوغ ، لذا فعلينا اعتبار هذه النتائج ناقوس خطر ضد استخدام الهواتف المحموله على نطاق واسع ومتزايد بين الذكور في سن المراهقة والبلوغ. وبالتالي، فيوصي و بشده على عمل المزيد من الدراسات حول هذه الفئة العمرية ومحاوله ايجاد الحلول الوقائية الممكنه.