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Effects of 50 Hz Magnetic Field on the Testes and Sperms of Adult Albino Rats

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Abstract: This study evaluates the 'effect of 50 Hz magnetic field' on the testicular gross morphology and histology, sperm morphology and meiotic chromosomes of adult Albino rats. The rats were divided into three groups; Control and two exposed groups to 0.15 mT and 20 mT. Thirty seven rats were included in each group. The exposure time lasted for 2 weeks, 3 days/week and 1/2 hour/day. After exposure, the rats were sacrificed by cervical dislocation and handled for evaluation. One of the testes was examined for gross morphological and histopathological changes. The other one was handled for evaluation of meiotic chromosomal abnormalities in the spermatocytes. The sperms were extracted from the epididymis and vas deferens for morphological investigation.

There were no observed gross morphological changes. The histopathological evaluation revealed a picture of maturation arrest at the level of the round spermatids with slight degenerative changes. These changes were more marked at the third group. The morphometric study showed reduction in the cellular surface area of the seminiferous tubules and an increase in the thickness of the basal lamina of seminiferous tubules, the 'number of the Leydig cells in the interstitial space' and the surface area of blood vessels. There was no effect on either the seminiferous tubular diameter, nucleo-cytoplasmic ratio of Leydig cells or the Sertoli cell number. The sperm morphological evaluation showed increase in the number of abnormal forms due to exposure. The chromosomal investigation showed also an increase in the chromosomal abnormalities after exposure.

Keywords: Chromosomes. magnetic field, sperm morphology, testicular gross.

INTRODUCTION

Normal and medical applications of low frequency magnetic field either as a diagnostic tool or therapeutic technique usually increase the chance of exposure to these fields [1]. For extremely-low-frequency fields, including those from the power lines, home appliances and wiring, the electric component can be easily attenuated, therefore magnetic field, which is not easily attenuated, is assumed to be the source of many possible health hazards [2]. Exposure to magnetic fields as reported, can lead to decrease in melatonin [3], testosterone and estrogen [4] hormones and might increase the effects of carcinogens [5] and reproductive male traits [6]. Although it was found that exposure to extremely low frequency magnetic field has no adverse effects on reproductive system of male rats [7], existence of certain frequency windows for the resonance of the influence of magnetic field on human spermatozoa was reported [8] and the continuous exposure to magnetic fields may induce the duration- and dose-dependent apoptosis of testicular germ cells [9].

'This study was undertaken to investigate the effects' of 50 Hz magnetic field of low (0.15 mT) and high (20 mT) intensities on the testicular gross morphology and histology, sperm morphology and meiotic chromosomes of adult Albino rats'.

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MATERIALS AND METHODS

'Adult male albino rats' aged 3 months old, to guarantee testicular maturity [10], were used in this study. The rats (Sprague-Dawely) were obtaied from the Breeding Unit, National Research centre, Cairo and received food and tap water ad libitum and housed in a proper control of lighting, humidity and temperature.

All the principles of the animal care concerning the ethical standards of animal handling and protection were followed carefully.

The animals were divided into three groups, each of 37 rats:

Group A: control (or sham) group in which the rats were exposed to the same used field but not energized.

Group B: the rats of this group were exposed to 50 Hz magnetic field of intensity 20 mT.

Group C: in which the rats were exposed to 50 Hz magnetic field of 0.15 mT.

The exposure time was 30 min/day, 3 times/week for 2 weeks [11]. The source of magnetic field was due to Helmholtz Coils, designed in The Electronic Centre, Cairo

Table 1. Gross morphological changes in the rat testes.

Group C (Mean ± SD)	Group B (Mean ± SD)	Group A (Control) Mean ± SD	Parameters
1.38 ± 0.1	1.4± 0.18	1.41 ± 0.19	Absolute testicular weight (gm)
0.61± 0.06	0.67± 0.07	0.62± 0.12	Crude testicular weight (gm)
1.74 ± 0.23	1.78 ± 0.16	1.76 ± 0.18	Testicular length (cm)
1.02 ± 0.09	1.01± 0.1	0.93 ± 0.21	Testicular width in cm

Absolute testicular weight represents the mean weight of both testes. Crude testicular weight represented1gm of testicular weight /100 gm of total body weight.

University. The diameter of the coils was 30 cm and of 250 turns. The field was probed by the flux meter ELWE 8533996 (Cerlingen, Germany). The magnetic field was firstly mapped in X, Y and Z- directions to choose the suitable region between the coils at which the field was approximately uniform. The region of exposure was capable of hosting a suitable movable and transparent cage located at the region where the field is approximately uniform. The material of the cage was chosen so that it did not alter the values of the field [12].

The exposure system was supplied by a cooling system to avoid any thermal effect.

The testes were evaluated for any gross abnormalities with recording its width, length, weight and its weight in relation to the total body weight. One testis from each rat was dissected. Dehydration, clearing and embedding in paraffin were carried out and 7 µm paraffin sections were cut serially. The longitudinal sections of rat testis were stained with hematoxylin and eosin (H&E) and with periodic acid schiff (PAS) [13].

Using a micrometer ocular lens, the following measurements were done:

diameter of the seminiferous tubules and their lumena with calculation of its cellular surface area.

thickness of the basal lamina of the seminiferous tubules.

diameter of the blood vessels with calculation of its surface area.

diameter of the Leydig cells and its nucleus with calculation of its nucleo-cytoplasmic ratio.

Also the following was done:

'counting the number of the Leydig cells in the interstitial tissue',

'counting the number of Sertoli cells per seminiferous tubule'.

All the data were detected in 10 different areas with the mean values recorded [14].

The rat sperms were collected from the vas deferens and epidydimes as they were excised and minced in 2 ml of 0.9% physiological saline, pipetted with 5ml pipette several times, filtered with silk mesh. A drop of the remnants were spread by a clean cover-glass over a clean slide then left in air to dry. The slides were stained with '1% Eosin-Y (aqueous)' [15]. For each rat, about 800 sperms were examined and 'morphological abnormalities' of sperm head, neck and tail were recorded according to the criteria. The results were statistically evaluated with Analysis Of Variance (ANOVA).

The testes were prepared according to [16]. The tubules of testes were squashed in isotonic solution (Sodium citrate 2.2%), centrifuged, then put in hypotonic solution (Sodium citrate 1.1%) and incubated at 37°C, then centrifuged, fixed and suspension spread on glass slides, stained with Giemsa stain and examined microscopically.

About 120 spread of Diakinesis- Metaphase I was examined for each group and chromosomal abnormalities were recorded. The results were statistically analyzed using Chi-square analysis.

RESULTS

1. Gross Morphological Changes

There were no significant changes in the gross morphological changes in the rat testes as can be seen in Table **1**.

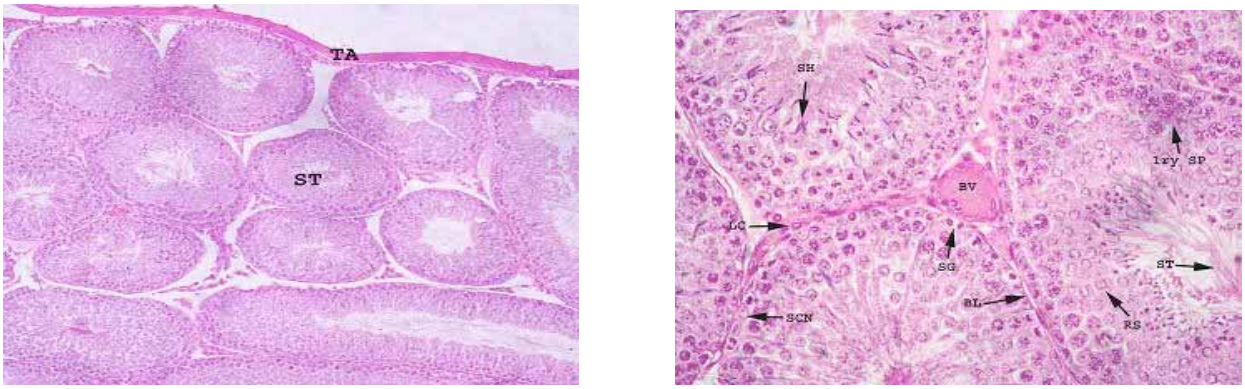
2. Histopathological Changes. (Hematoxylin and Eosin (H&E) Sections)

Control Group (A); the Rats are Exposed to Sham Field

The longitudinal sections in the testes of the control group showed rounded, oval and longitudinal seminiferous tubules (ST) packed together within a thick fibrous tunica albuginea (TA). Each seminiferous tubule was covered by thin basal lamina. The seminiferous tubules showed different cellular stages of maturation starting with the spermatognia in its two types A &B. Between spermatogonia, there are Sertoli cells which represent the main supportive and nutritive cells in the seminiferous tubules. Next to the spermatogonia come the spermatocytes – 1ry and 2ry- then come the spermatids with rounded (early) form and longitudinal (late) one. The sperms are found to fill almost the whole tubular lumen (Fig. **1a**, **b**).

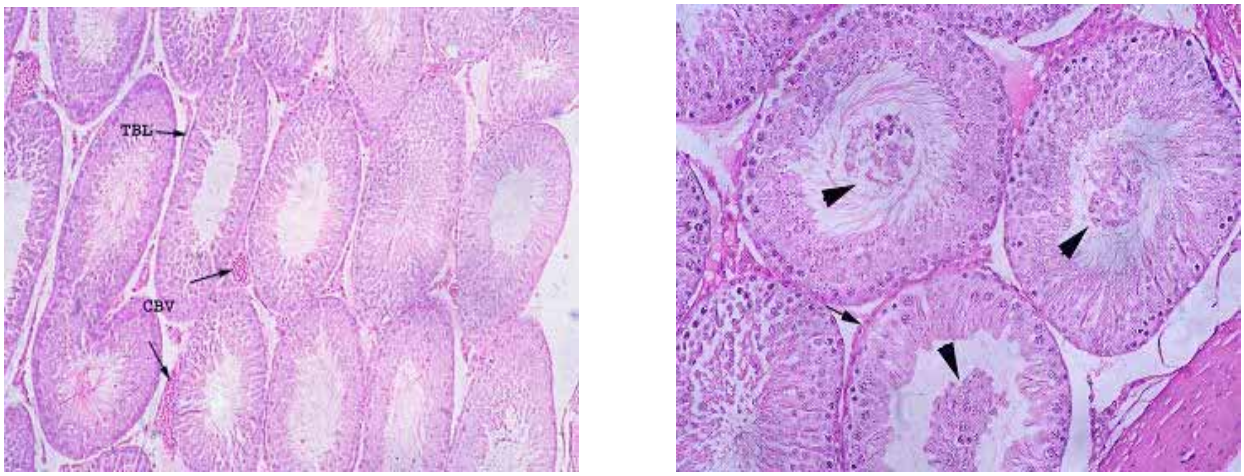
Group (B); the Rats are Exposed to 0.15 mT

The testes in this group showed a maturation arrest in most of the seminiferous tubules at the early (rounded) spermatidswith neither further progression nor sperm development.

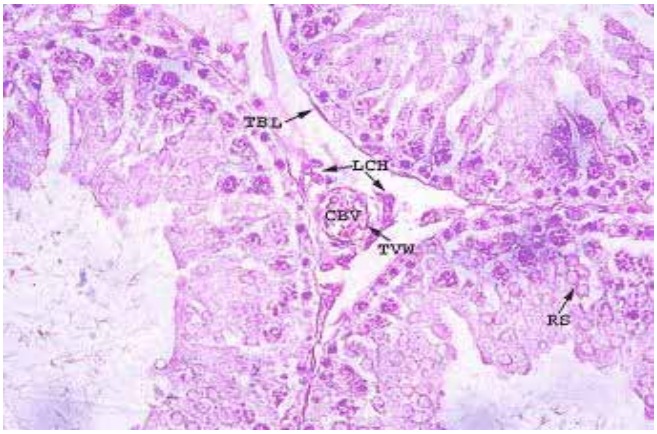


(a) **(b)**

Fig. (1). a) Normal architecture of the rat testes taken from control group. (H&E × 100) TA= tunica albuginea, ST= seminiferous tubule. **b**) Section in the rat testes of control group showing different types of germinal epithelium and supporting stroma. (H&E × 400). SG= Spermatogonia, 1ry SP= Primary spermatocytes, RS= Rounded spermatids, SH= Sperm heads, ST= Sperm tails, LC= Leydig cells, BL= Basal Lamina, BV= Blood vessel, SCN= Sertoli cell nucleus.

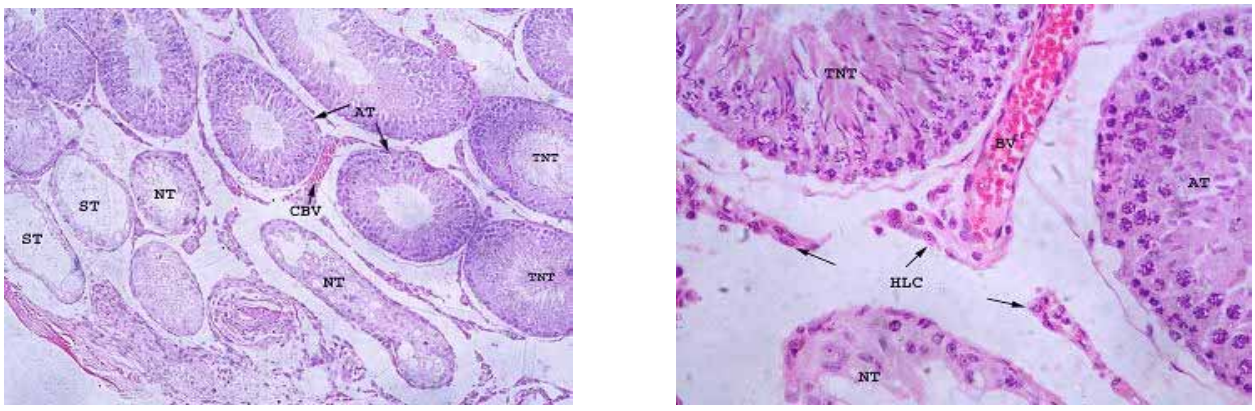


(a) **(b)**



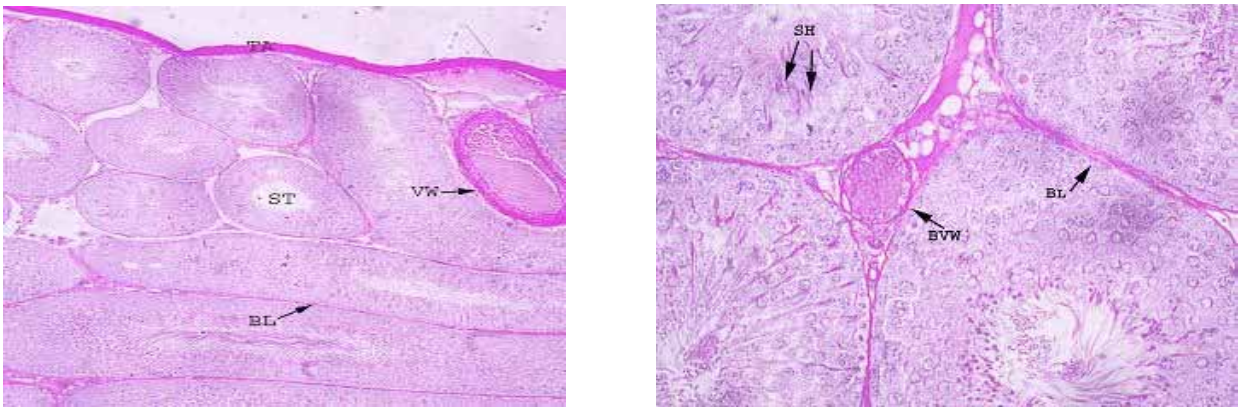
(c)

Fig. (2). a) Section from rat testes exposed to low intensity magnetic field with thickened basal lamina (TBL) and congested blood vessels (CBV). (H&E × 100). **b**) Section in the rat testes of Group B displaying desquamation of the immature cells in the lumen of the seminiferous tubules (arrow heads). The arrow points to the thickened basal lamina. (H&E ×200). **c**) Section in the rat testes of Group B showing the overall arrest of the tubules at the rounded (early) spermatid (RS) stages of development and the reduction in the cellularity of the seminiferous tubules (H&E ×400). LCH= Leydig cell hyperplasia, TBL= Thickening in the basal lamina, TVW= Thickening in the vessel wall, CBV= Congestion in the blood vessel.



(a) **(b)**

Fig. (3). a) Section in the rat testes of the high intensity group showing (AT= arrested tubules), (NT= necrotising tubules), (ST= shadow tubules), totally normal tubules (TNT) just beside the affected ones with congested blood vessels (CBV). (H&E ×100). **b)** Section in rat testes of Group C with the presence of arrested tubules (AT) and necrotizing tubules (NT) beside the totally normal tubules (TNT). Note the marked hyperplasia of the leydig cells (HLC) beside the congested blood vessel (BV). (H&E ×400).



(a) **(b)**

Fig. (4). a) Section in rat testes of group A with positive PAS reaction of the tunica albuginea (TA), basal lamina (BL) of the seminiferous tubules (ST) and vessel wall (VW). (PAS ×100) **b)** Section in rat testes of group A with positive PAS reaction of the heads of the sperms (SH), basal lamina of the seminiferous tubules (BL) and blood vessel wall (BVW). (PAS ×400).

Another change was detected represented in the form of desquamation of some cells in the lumen of the seminiferous tubules indicating an early degenerative change (Fig. **2a**, **b** and **c**).

Group (C); the Rats are Exposed to 20 mT

In this group, the maturation arrest was much more marked among the seminiferous tubules. Degenerative changes were in a more frank picture showing necrotic tubules which progressed to totally lost tubules. Surprisingly among all the degenerative and maturation arrested tubules, some totally normal tubules could be detected in-between (Fig. **3a**, **b**).

3. Periodic Acid Shift (PAS) Sections

Control Group (A); the Rats are Exposed to Sham Field

The magenta discoloration was more marked at the tunica albuginea, basal lamina of the seminiferous tubules and inside the seminiferous tubules due to the presence of the acrosomal caps of the sperms with its crescentic shapes (Fig. **4a**, **b**).

Group (B); the Rats are Exposed to 0.15 mT

There was an increase in the PAS reaction in the basal lamina due to its thickening with loss of the PAS reaction in seminiferous tubules due to the maturation arrest and absence of acrosomal caps of the sperms (Fig. **5a**, **b**).

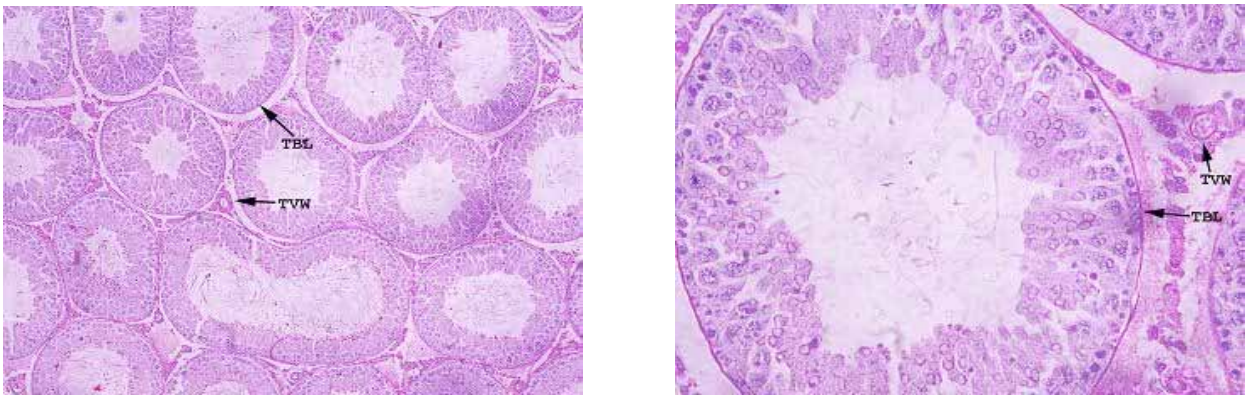
Group (C); the Rats are Exposed to 20 mT

There is an overall reduction in the PAS reaction in the seminiferous tubules of this group due to arrest of germinal epithelium and loss of sperms and its acrosomal caps with increase in the PAS reaction in the basal lamina due to its thickening (Fig. **6a**, **b**).

4. Morphometric Measurements

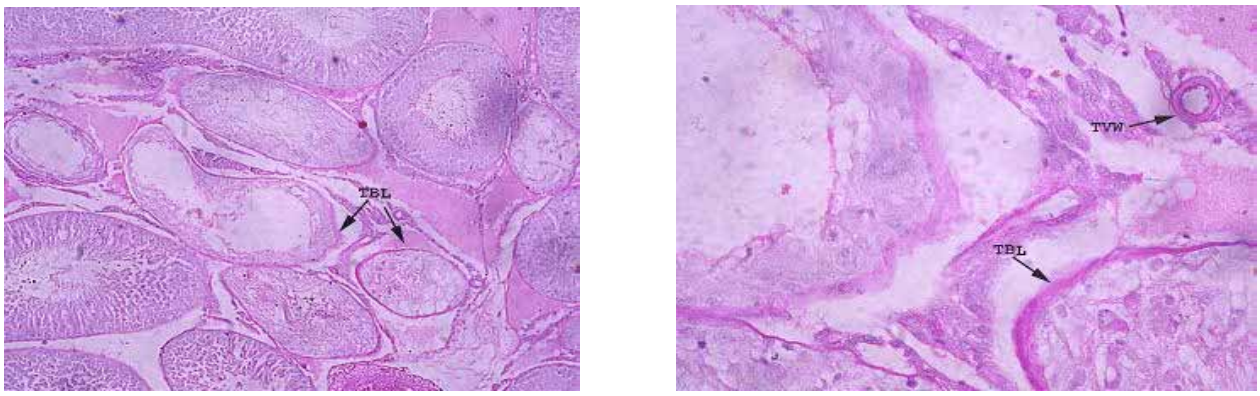
4.1. Cellular Surface Area

The mean cellular surface area of the testes was found to be decreased in both exposed groups to low (Li) {0.59 ± 0.11 mm^2} and high intensity (Hi) {0.57 ± 0.11 mm^2} or groups B and C respectively if compared to the control group A {0.6 ± 0.11 mm^2} as Fig. (**7a**) illustrates.



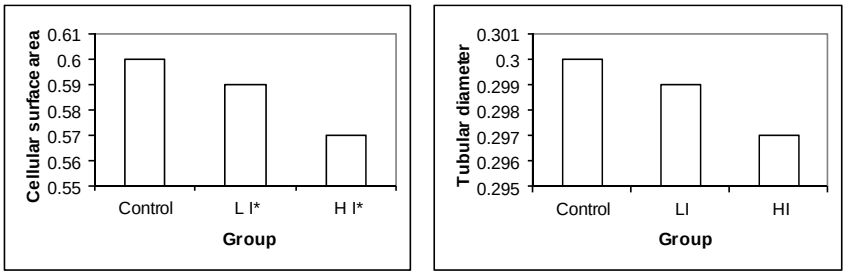
(a) **(b)**

Fig. (5). a) Section in rat testes of group B with increase in the +ve PAS reaction in basal lamina of the seminiferous tubules (TBL) and vessel wall (TVW) due to increase in their thickness. (PAS ×100). **b)** Section in rat testes of group B with increase in the +ve PAS reaction in the basal lamina of the seminiferous tubules (TBL) and vessel wall (TVW) with reduction in the PAS reaction in the seminiferous tubules due to absence of the sperm heads. (PAS×400).



(a) **(b)**

Fig. (6). a) Section in rat testes of group C with increase in the positive PAS reaction in the basal lamina due to increase in its thickness (TBL). (PAS ×100). **b)** Section in rat testes of group C with increase in the positive PAS reaction due to basal lamina thickening (TBL) and thickened vessel wall (TVW). (PAS ×400).



a **b**

Fig. (7). a) The mean cellular surface area of testes (mm^2) in the three studied groups($p<0.05$). **b)** The mean tubular diameter (mm) in the three groups.

4.2. Tubular Diameter

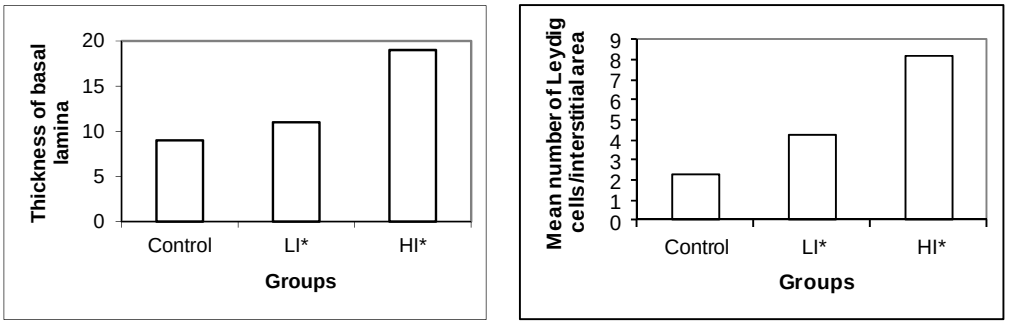
The mean tubular diameter also decreased, but not significantly, from (0.3 ± 0.038 mm) in control group to (0.299 ± 0.029 mm) in the low intensity group and reached (0.297 ± 0.033 mm) in the high intensity one as seen in Fig. (**7b**).

4.3. Thickness of the Basal Lamina

Fig. (**8a**) shows that the thickness of the basal lamina of the seminiferous tubules increased as a result of exposure from 9±2.6 µm (in control) to 11±3 µm (in low intensity group) and 19± 9.9 µm (in the high intensity one).

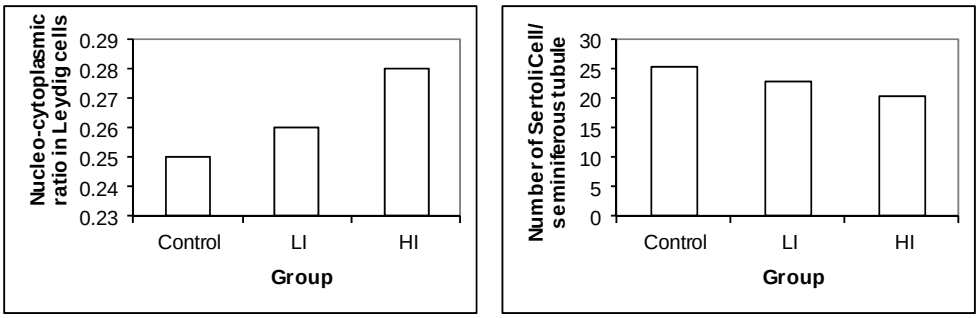
4.4. Number of Leydig Cells

The mean number of 'Leydig cells/ interstitial tissue' between the seminiferous tubules also increased from (2.3±0.56 cell/interstitial tissue) in control group to (4.3 ±1.18 cell / interstitial tissue) in low intensity group and reached (8.2±2.46 cell/interstitial tissue) in the high intensity one (Fig. **8b**).



a **b**

Fig. (8). a) The thickness of the basal lamina of seminiferous tubules (in μm) of the three groups. ($p<0.05$). **b)** The mean number of the Leydig cells/interstitial area in the three groups. $P<0.05$.



a **b**

Fig. (9). a) The nucleo-cytoplasmic ratio of Leydig cells in the three groups. **b**) The mean number of Sertoli cells /seminiferous tubule, $p>0.05$.

4.5. The Nucleo-cytoplasmic Ratio

There was an increase, but not significant, in the nucleo-cytoplasmic ratio in Leydig cells from control (0.25) to low intensity (0.26) and high intensity one (0.28) (Fig. **9a**).

4.6. Number of Sertoli Cells

The mean numbers of Sertoli cells/seminiferous tubule in the three examined groups were 25.4±7.7, 22.9±7.3 and 20.4±3.7 cell/seminiferous tubule respectively (Fig. **9b**).

4.7. The Surface Area of Blood Vessles

The 'surface area' of the blood vessels increased from 0.15±0.08 mm^2 (control) to 0.23±0.12 mm^2 and 0.29±0.19 mm^2 in low and high exposed groups respectively (Fig. **10**).

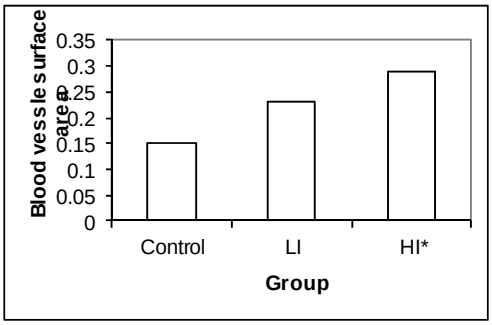


Fig. (10). The mean surface area of the blood vessels in mm^2. ($p< 0.05$).

5. Sperm Morphology

The results showed that about 3% of the total examined control sperms were deformed while 15% and 19% of the total numbers of Groups B and C respectively were deformed. Fig. (**11**) shows the normal structure and shape of rat sperm. Table **2** represents the total number of deformities with the values of the different forms and its statistical point of view. This table shows that head abnormalities for example, recorded 4.26±3.89, 15.76±14.13 and 24.64±16.03 in control, low intensity exposed group and high intensity exposed one, respectively, having 'significant difference' from the statistical point of view ($p<0.05$). These head abnormal-lities are also shown in (Fig. **12**), a, b and c. There were also

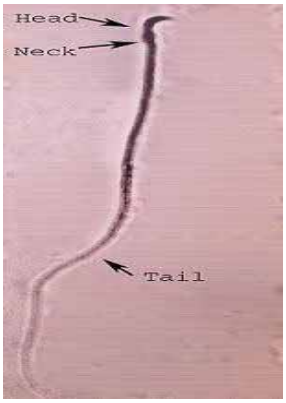


Fig. (11). Photomicrograph of sperm of the control group displaying normal shape of the rat sperm. (Eosin × 400).

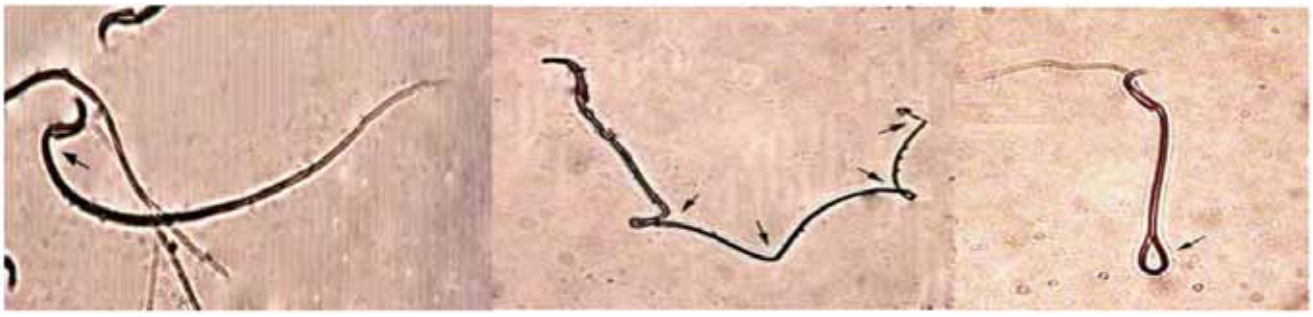
Table 2. The mean number of different abnormalities detected in rat sperms among the study groups.

High Intensity Group No./800 Sperm	Low Intensity Group No./800 Sperm	Control No./800 Sperm	Abnormality Detected	
2.63±8.21*	9.75±8.28*	2.13±1.89	Flat head	Head abnormalities
3.88±2.3*	1.63±1.51	1.25±1.16	Double head	
8.13±5.52*	4.38±4.34	0.88±0.84	Deformed head	
24.64±16.03*	15.76±14.13*	4.26±3.89	Total	
34±21.12*	33.38±24.86*	6.75±5.06	Bent neck	Neck abnormalities
59.75±46.65*	42.38±29.16*	8.63±5.95	Angulated tail	Tail abnormalities
21.88±11.56*	18.88±11.17*	1.5±1.41	Coiled tail	
81.63±58.21*	61.26±40.33*	10.13±7.36	Total	
22.88 ±12.49*	8.88 ±7.54*	1.88±1.76	Amorphous▪	
148.5±76.04*	117.5±64.29*	23.88±13.15	Total	

The data are represented with mean ±SD.
* Statistically significant P value for ANOVA of the group in relation to the control.
▪ Amorphous represented sperms with marked abnormalities in shape.

(a) **(b)** **(c)**

Fig. (12). Examples of the deformed head in rat sperms due to magnetic field.



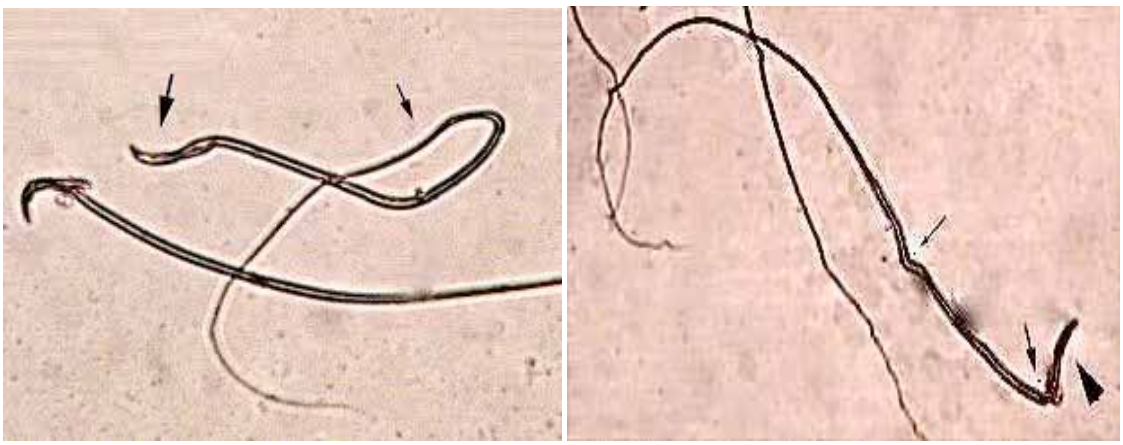
(a) **(b)** **(c)**

Fig. (13). Sperm deformities **a**) bent neck, **b**) angulated tail and **c**) coiled tail.

deformities in the neck and tail of the sperms as (Fig. **13**) shows. Amorphous sperms were also observed (Fig. **14**) showing frequencies of 1.88±1.76 in control group, 8.88±7.54 in low intensity exposed group and 22.88±12.49 in high intensity exposed one '($p<0.05$)' as Table **2** illustrates.

6. Chromosomal Abnormalities

The total number of chromosomal abnormalities showed statistically significant differences ($P<0.05$) detected in the exposed A and B groups (34.5% and 61.7% respectively) if



(a) **(b)**

Fig. (14). a & **b** amorphous rat sperms.

Table 3. The percentage of different chromosomal abnormalities detected in the studied groups.

HI	LI	Control	Abnormalities
19.3%*	11.7%*	0.9%	Chromosomal breaks
11.7%*	8.5%	1.6%	Chromosomal gabs
14.9%*	5.7%	0.9%	Chromosomal fragment
8.2%*	5.7 %*	0%	Autosomal chromsomes univalents
3.2%	2.2%	0%	Sex chromosomes univalents
0.6%	0%	0%	Transloacation chain III + Autosomal Univalent
0.9%	0%	0%	Translocation chain IV
0.9%	0%	0%	Translocation ring IV
0.9%	0%	0%	Polyploidy
0.9%	0.6%	0%	Sticky chromosomes
61.7%*	34.5%*	3.8%	Total number of abnormality

The Data in the schedule are represented with % of the total number of abnormality.
*Statistically significant P value for the X^2 test of the group in relation to the control ($p<0.05$).

compared to the control A group (3.8%) as Table **3** illustrates.

The different chromosomal abnormalities rat in the diakinesis-metaphase I (if compared to the control, Fig. **15**) can be also shown in (Figs. **16**-**18**).

DISCUSSION

It was reported that 15% of all couples of reproductive age have difficulty in achieving pregnancy and about 50% of fertility problems are may be due directly or indirectly to male reproductive disorders [17]. Because of the high mitotic rate of germ cells, the testes are a vulnerable organ when exposed to static or time varying magnetic fields [18].

In this study, the testicular morphological parameters showed no significant changes after exposure to magnetic field. Long period exposure of testes to magnetic field could produce a decrease in diameter of the reproductive ducts and the weight of testes [19].

The results also showed testicular histopathological changes after exposure to high magnetic field. These changes, as reported, were also found due to exposure to 20 mT and could be induced by L-carnitine [20]. Maturation arrest in most seminiferous tubules was detected in this study. This might result from insufficient numbers of mature spermatids and testicular degeneration [21].

Chromosomal and genetic abnormalities affecting meiosis have been implicated in some cases of male infertility associated with maturation arrest. The defective meiosis seen in maturation arrest may result from chromosomal translocations, desynapsis, or reduced chiasma formation [22]. Loss of germinal epithelial cells results in

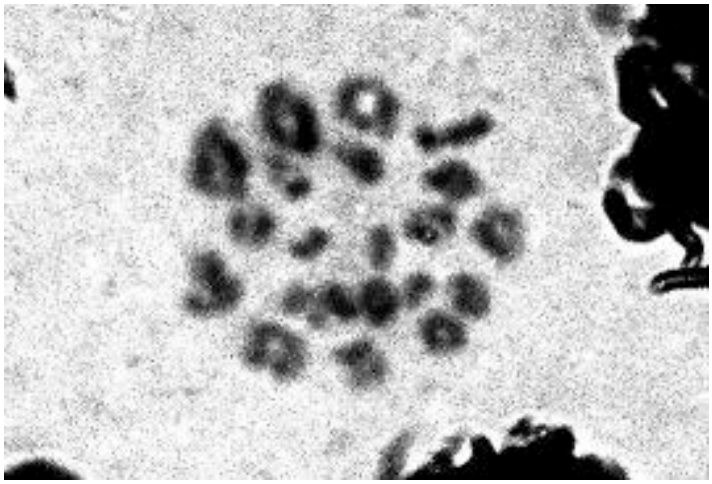
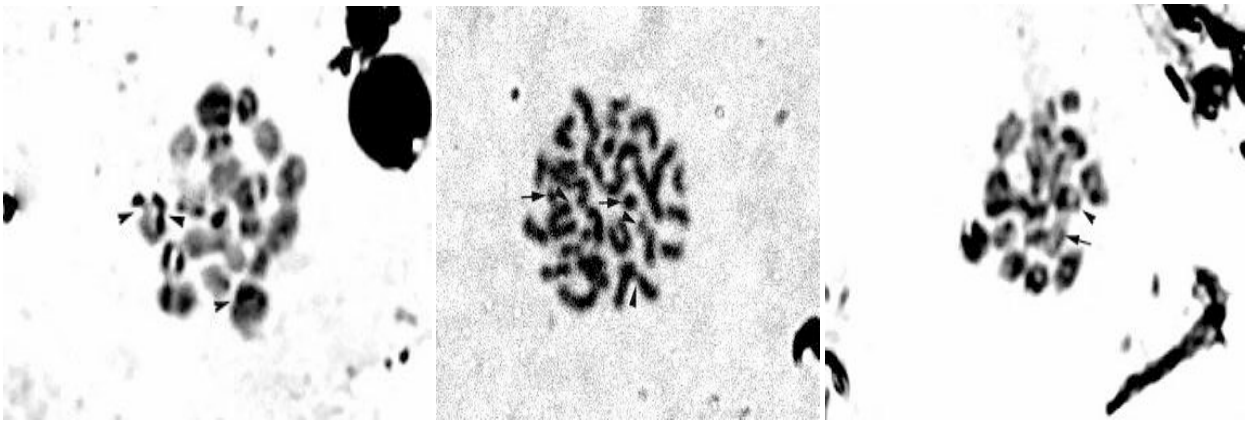
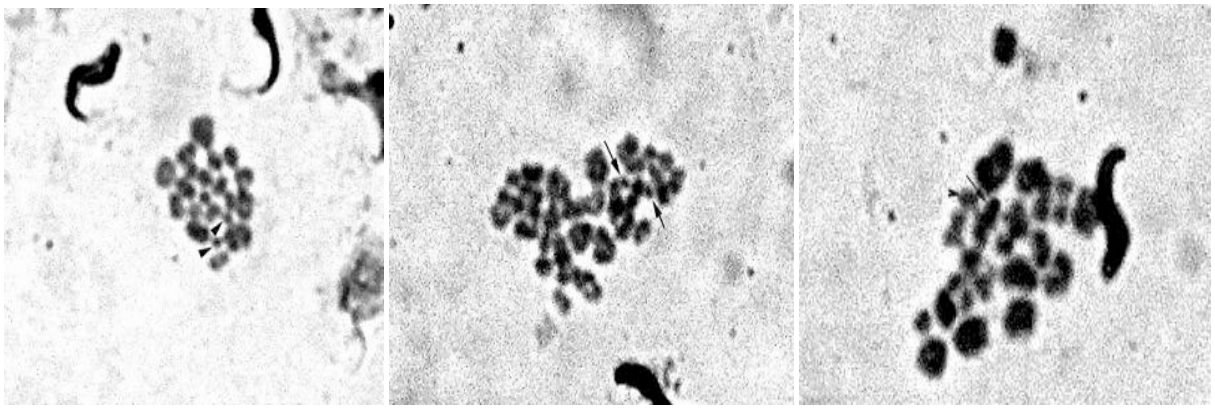


Fig. (15). Normal meiotic spread of the rat taken from control group. (Giemsa × 400).



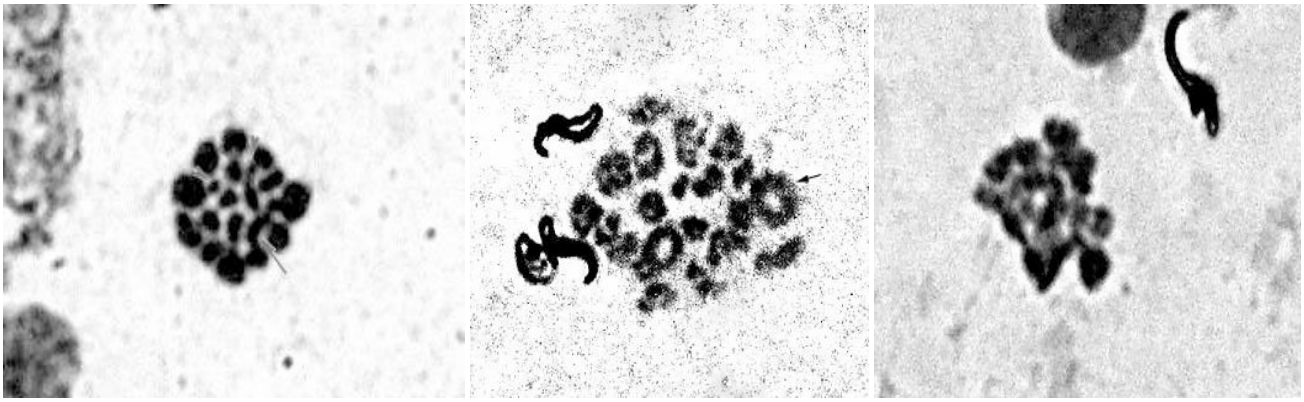
(a) **(b)** **(c)**

Fig. (16). a) Multiple gab abnormality, the arrow heads point to chromosomal gabs in the diakinesis-metaphase I. **b**) Break and fragment abnormality, arrow heads point to breaks while arrows point to fragments. **c**) Sex univalent abnormality, arrow points to X chromosomes, while arrow head points to Y chromosome.



(a) **(b)** **(c)**

Fig. (17). a) Autosomal univalent abnormality, Arrow heads point to autosomal univalent. **b**) Autosomal Univalent abnormality (Arrows) with polyploidy. **c**) Translocation chain abnormality, Arrow points to chain trivalent (CIII) while arrow head points to autosomal univalent.



(a) **(b)** **(c)**

Fig. (18). a) Translocation chain IV abnormality, Arrow points to translocation chain IV, while arrow heads point to autosomal univalent abnormality. **b)** Translocation ring abnormality. **c)** Sticky chromosomal abnormality.

apparent dilatation of the seminiferous tubules which may be attributed to maturation arrest [21] as measuring the tubular diameter in the studied groups did not reveal any statistically significant change in it.

This was also accompanied by Leydig cell hyperplasia and congestion in blood vessels that led to increase in the vessels surface area [18]. Similarly, decreased spermatogenesis in some seminiferous tubules, congestion was found in blood vessels of the interstitium and an increase in interstitial edema and Sertoli cells [23]. A significant increase in the incidence of germ cell death with a decrement in the number of well organized seminiferous tubules was also observed [24]. Also a significantly higher apoptotic rate with exposure to electromagnetic field maturation arrest was limited to the elongated spermatids [25].

The number of Sertoli cells was not much affected in this study since there was a minimal reduction in the number of them/seminiferous tubule. The histopathological changes were more marked in the high intensity exposed group as also recorded where exposure of mice to magnetic field intensity of 20 mT led to marked histopathological changes represented in wide tubular dilatation, sloughed lining and absent spermatogonia and spermatogenesis [11].

The nucleo-cytoplasmic ratio of the Leydig cells also increased. Although this was not statistically significant, but this may suggest that the nuclear activity of Leydig cells was enhanced [26]. The experimental disruption of spermatogenesis was associated with hyperplasia and hypertrophy of the Leydig cells which may be attributed to variable and still poorly understood in the human male [27]. The fertilizing capacity of abnormal spermatozoa was found to be controversial and certain defects in spermatozoa were accompanied by infertility e.g. coiled tail in bull sperms. This study showed that the mean number of abnormality/800 rat sperm increased in due to exposure to magnetic field. The difference was statistically significant ($p<0.05$). This was in consistence with [28]. The most frequent deformity detected in the studied groups was the angulated sperm with a highly statistical significant difference in the three groups ($p<0.01$) followed by coiled tail sperms with also $p<0.005$). The bent neck and flat head were also observed more frequently in mice exposed to magnetic field [17, 29].

Working with the fertility issue has made much concern to the study of meiotic chromosomes. Several studies noted that the incidence of chromosomal abnormalities among males attending fertility clinics rises as the sperm count declines [30].

The present study found that there was an increase in the chromosomal abnormality as a result of exposure to magnetic field. The most frequent recorded abnormality was the chromosomal break ($p< 0.05$) followed by chromosomal gab and fragments. On the other hand, no statistically significant difference in meiotic chromosome aberrations in spermatocytes of rats exposed to 60 Hz magnetic field was detected [31]. Semen samples from healthy men exposed to extremely low frequency electromagnetic fields were with no significant difference in the incidence of chromosomally abnormal spermatozoa between the exposed and the control groups [32].

Autosomal univalents were only recorded in the exposed groups (low and high intensity ones) in this study; sex univalents were in both exposed groups with non statistically significant difference.

Different forms of reciprocal translocations have been also detected in this study. Translocation ring of four figures (ring IV), translocation chain of four figures (chain IV) and chain three plus one univalent figure (chain III+I). Reciprocal translocations have been a phenomenon limited to the high intensity group. In the mouse a positive correlation has been noted between severity of effects on the sperm count and percentages of the spermatocytes showing chain configurations at meiosis. Thus, as the proportion of cells with chains rises, the sperm count is reduced [33].

In conclusion, exposure to magnetic field either low intensity or high intensity values may affect to a considerable degree, the histology, sperm morphology and meiotic chromosomes of the rat testes.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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