Role of bone marrow derived mesenchymal stem cells in protection of spermatogenic and Sertoli cells against histological alterations induced by torsion/detorsion in rats

Original Article

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ABSTRACT

Background and Objectives: Testicular torsion is an emergency. Thus, early diagnosis and appropriate treatment are fundamental to avoid irreversible testicular damage. The present study aimed to throw more light on the histological alterations in adult albino rat's ipsilateral testis after unilateral testicular torsion and detorsion (T/D) and to demonstrate the effect of mesenchymal stem cells (MSCs) injection on these alterations.

Materials and Methods: Twenty seven adult healthy male albino rats were divided into 3 main equal groups: Group I (Control group), Group II (T/D): the rats were surgically operated to perform T/D and Group III (T/D+MSCs): the animals were injected with MSCs in tail vein at the end of the surgery. All rats were sacrificed ten weeks from the start of the experiment and testis specimens were processed to be examined by light and electron microscope. Blood specimens were taken to measure serum testosterone level.

Results: Degenerative changes were observed in spermatogenic and Sertoli cells of T/D group and were associated with statistical significant reduction in serum testosterone level, height of germinal epithelium, proliferating cell nuclear antigen (PCNA) and vimentin immunoexpression. These changes were observed to be reduced in T/D+MSCs group.

Conclusions: MSCs treatment could protect the spermatogenic and Sertoli cells from degenerative changes in testis after T/D in adult albino rats. Hence, MSCs therapy could be considered as a promising therapeutic tool to preserve spermatogenesis in cases of testicular T/D and should be more studied either on experimental animals or human.

Key Words: BM-MSCs, PCNA, sertoli cell, spermatogenic cell, torsion, vimentin

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INTRODUCTION

Torsion occurs as the result of rotation of testis around the spermatic cord between 90° and 180° leading to cut off blood flow to and from the testis with ischemia to the ipsilateral testis and complete torsion usually occurs when the testicle twists 360° or more[1]. The chance for testicular viability after torsion was significantly decreased after 6 hours from the onset of symptoms and if 24 hours or more pass, testicular necrosis develops in most of the patients. Hence, the early diagnosis and treatment are important to save the testis and to preserve the fertility[2].

Bone marrow derived mesenchymal stem cells (MSCs) are examples of adult stem cells which are preferred in stem cell research due to their ability to proliferate extensively and to differentiate into multiple cell types[3]. MSCs possess a great therapeutic potential and their beneficial effect in injured tissues is attributed to their differentiation, activation of a protective mechanism and stimulation of endogenous regeneration[4]. The testis of infertile rats was reported to accept the transplanted MSCs which could differentiate into all types of germinal cells in testicular seminiferous tubules[5].

Proliferating cell nuclear antigen (PCNA) is an essential component of the DNA replication regulator, serves as a co-factor for DNA polymerase delta in S-phase and is required for DNA recombination and repair. PCNA encircle the DNA and acts as a scaffold to recruit proteins involved in DNA replication, DNA repair and chromatin remodeling[6].

Vimentin is an intermediate filament protein expressed in Sertoli cells providing them structural and functional support[7]. So, the study of vimentin expression could be useful to understand the changes in Sertoli cells.

Considering the previous reports, the present work was designed to throw more light on the histological alterations in adult albino rat's ipsilateral testis after unilateral testicular torsion/detorsion and to demonstrate the effect of MSCs injection on these alterations.

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PATIENTS AND METHODS

I. Stem cells

Bone marrow derived-mesenchymal stem cells (BM-MSCs) were provided from Biochemistry Department, Faculty of Medicine, Kasr Al-Ainy Medical School.

II. Experimental animals

Twenty seven adult healthy male albino rats (150–200 grams body weight) were used in this study. They were obtained from breading animal house, Faculty of Medicine, Zagazig University. Experimental protocol was approved by the Zagazig University Research Ethics Committee. The animals were kept in the animal house for one week in stainless steel cages to be acclimatized to the new environment before the experiment. The animals were maintained in accordance with the guidelines of stem cell research unit in the central laboratory, Zagazig University. Throughout the duration of the experiment, the rats were housed at room temperature with normal light/dark cycles and were allowed ad-libitum access to food and water.

The rats were divided into 3 main equal groups (nine animals for each group). Group I (control group): The rats were subdivided into three subgroups (3 animals each): Subgroup Ia (negative control): The rats received no treatment. Subgroup Ib (positive control): These rats received 0.5ml of phosphate-buffered saline (PBS), as a vehicle of stem cells, injected in the tail vein. Subgroup Ic (sham-operated): The rats had undergone sham operation. Group II (torsion/detorsion group): the rats had undergone surgical operation through which torsion/detortion (T/D) was performed. Group III (torsion/detorsion + MSCs treatment) the rats had undergone the same surgical operation as in group II then, immediately at the end of the surgical procedure, the animals were injected with undifferentiated MSCs prepared from bone marrow of male albino rats at a dose of 1×10^6 cells per rat suspended in 0.5ml of phosphate-buffered saline injected in the tail vein[8]. The rats of the all groups were sacrificed ten weeks after the operation[9].

III. Surgical procedures:

All surgical procedures were performed under aseptic conditions. The animals were anesthetized using intraperitoneal pentobarbital injection in a dose of 4mg/100 gm body weight[10].

Sham operation:

The scrotum was opened through a midline incision and the tunica vaginalis was opened then the left testis was delivered to the surgical field. The testis was fixed to the scrotum with 40/ silk sutures and then the incision was closed without applying torsion. After 2h, the incisions were reopened and the silk sutures of the testes were removed[11].

Torsion/detorsion:

The scrotum was opened through a midline incision and the tunica vaginalis was opened then the left testis was rotated 720° in a clockwise direction and maintained in this torsion position by fixing the testicle to the scrotum with 40/ silk sutures and then the incision was closed. The animals were allowed to receive butorphanol (30 mg/kg) subcutaneously for postoperative analgesia. After 2h, the incisions were reopened and detorsion was made and testes were returned to their original position in the scrotum and the incision was closed with the silk suture[11].

IV. Methods:

Light microscope technique:

At the end of the experiment, the animals of all groups were anaesthetized by ether and then left testes were dissected and their capsules were opened carefully and immersed in fixative 10% formol-saline. After 10 minutes, until testicular tissue slightly hardened, 1 cm3 specimens were fixed and processed to prepare paraffin blocks. Sections of 5-µm thickness were cut and stained with Hematoxylin and eosin (H&E) stains[12]. Immunohistochemical procedure was carried out using an avidin– biotin–peroxidase complex technique to detect proliferating cell nuclear antigen (PCNA) in the nuclei of spermatogenic cells and to detect vimentin, an intermediate filament protein, in Sertoli cells. The deparaffinized sections (4-µm thickness) were incubated in 0.3% hydrogen peroxide for 30 min to block the endogenous peroxidase. After rinsing with phosphate buffer saline (PBS), sections were incubated with the primary antibodies against PCNA or vimentin in a moist chamber. Anti-PCNA mouse monoclonal antibodies were used in dilution of 1:2000 (Cat.# sc-56, Santa Cruz Biotechnology) and anti-vimentin mouse monoclonal antibodies in dilution of 1: 400 (Cat.# M7020, Dako) for 60 minutes at room temperature. Then the slides were washed with PBS and incubated with the secondary anti-mouse antibodies universal kits obtained from Zymed Corporation. Staining was completed by incubation with substrate chromogen DAB (3,3 diaminobenzidine) for 13- minutes which resulted in brown-colored precipitate at the antigen sites and Mayer's hematoxylin was used as a counter stain. Positive control was skin and negative control sections were prepared using PBS without using the primary antibody[13]. Sections were viewed using an Olympus microscope (C5060-AUD, 5H01155 JAPAN) and images were captured by a digital camera (Canon PowerShot A620, England, UK). The scale bar was added to the photomicrographs according calibration sheet using power point (https://www.youtube.com/watchv=3bynnPQQxmg).

Transmission electron microscope technique:

Specimens (1 mm^3) from left testes were immediately fixed in 2.5% glutaraldehyde buffered with PBS at pH 7.4

for 2 h at 4°C and postfixed in 1% osmium tetroxide in distilled water for 1 h at 4°C, dehydrated, and embedded in epoxy resin. Semithin sections (0.5 µm) were stained with toluidine blue stain and ultrathin sections (70-90 nm) were stained with uranyl acetate and lead citrate[14]. The ultrathin sections were examined and photographed using a JEOL-JEM 1010 electron microscope (Jeol Ltd, Tokyo, Japan) in the Histology Department, Faculty of Medicine, Zagazig University, and a JEOL JEM 2100 EXII Electron Microscope (Jeol Ltd), Electron Microscope Research Laboratory, Faculty of Agricultural, Mansoura University, Egypt.

V. Morphometric study:

The height of germinal epithelium was measured using H&E stained sections from photos of X 200 magnification by Digimizer 4.3.2 (MedCalc Software bvba, Belgium) from randomly selected three different fields/rat of each group. From anti-PCNA and anti-vimentin immunohistochemically stained sections, the areas of positive nuclear PCNA and cytoplasmic vimentin immunoexpressions, respectively, were done within 6 fields for each rat at a total magnification ×200 using image J image analysis software (National Institute of Health; NIH, Bethesda, MD, USA)[15]. In each chosen field, the germinal epithelium lining the seminiferous tubules were enclosed inside the standard measuring frame. The positively reactive areas were masked by a red binary color to be measured and the total area (in %) of positive immunoreactions in each photo was measured and their mean values for each group were obtained.

VI. Hormonal analysis.

Venous blood (4 ml) was collected in a capillary tube by retro- orbital puncture from all rats of each group before sacrifice. Blood samples were prepared to measure serum testosterone level and the values expressed as pg/ ml[16].

VI. Statistical analysis.

Statistical analysis was performed for the serum level of testosterone, height of germinal epithelium and the area percentage of PCNA and vimentin. All values of the experiments were represented as mean ± Standard Deviation (SD). One-way analysis of variance (ANOVA) was used, followed by Post hoc least significant difference (LSD) test to evaluate the differences between the groups. For all comparisons $P<0.05$ were considered as significant difference. All analyses were performed using the IBM SPSS 19.0 software.

RESULTS

The histological, ultrastructural, immunohistochemical and biochemical studies of control subgroups Ia, Ib and 1c showed the same results thus, was chose subgroup Ia as the control group in the results.

I- Light and electron microscope results:

Light microscopic examination of testis sections from control group showed seminiferous tubules with regular outlines and lined by Sertoli cells, spermatogonia, primary spermatocytes, early spermatids, late spermatids and sperms. Sertoli cells appeared with large pale oval nucleus and prominent nucleolus. Interstitial cells of Leydig were also seen (Figs. 1A&B).

Electron microscopic examination of testis sections from control group showed Sertoli cells with pale euchromatic indented nuclei and prominent nucleoli. Tight junction of blood testis barrier was present between processes of Sertoli cells with its bundles of microfilaments appearing in its cross section. Spermatogonia appeared with rounded nuclei exhibiting peripheral heterochromatin clumps. Primary spermatocytes had large rounded nuclei, condensed mitochondria with few cristae and free ribosomes (Figs. 2A&B). Early spermatids appeared with rounded euchromatic nuclei, numerous peripherally arranged mitochondria, prominent Golgi cisternae and acrosomal caps on one side of the nucleus and late spermatids contained elongated condensed nuclei. The basement membrane of the seminiferous tubules was surrounded by thin myoid cell (Fig. 2C).

Light microscopic examination of testis sections from torsion/detorsion (T/D) group showed some shrunken seminiferous tubules with irregular outlines separated by wide interstitium (Fig. 3A). Some exfoliated germ cells and areas of homogenous acidophilic material within germinal epithelium and within the interstitium were observed. Areas of germ cells loss, giant cells and detached spermatogonia from underlying basement membrane were also detected (Figs. 3B-E).

Electron microscopic examination of testis sections from T/D group showed some Sertoli cells with small nuclei and multiple vacuoles (Fig. 4A). Irregular spermatogonia detached from both the basal lamina and from the electron dense cytoplasmic processes of Sertoli cells with loss of blood testis barrier were observed (Figs. 4B&C). Spermatids with eccentric nuclei, distorted acrosomal caps, vacuoles and electron dense bodies were detected with presence of wide intercellular spaces (Fig. 4D).

Light microscopic examination of testis sections from torsion/detorsion treated with MSCs (T/D + MSCs) group showed apparently normal seminiferous tubules lined by preserved germinal epithelium with some widened intercellular spaces and narrow interstitium (Figs. 5A&B).

Electron microscopic examination of testis sections from T/D + MSCs group showed Sertoli cells with euchromatic nuclei, prominent nucleoli and cytoplasmic electron dense bodies (Fig. 6A). Some mitochondria with destroyed cristae were observed in the cytoplasm

of spermatogonia and Sertoli cells. Preserved junction of blood testis barrier between Sertoli cells was noticed (Fig. 6B). Preserved early spermatids with rounded euchromatic nuclei, peripherally arranged mitochondria, well-formed acrosomal cap and Golgi apparatus were observed. Few widened intercellular spaces, electron dense bodies and heads of late spermatids were also seen (Fig. 6C).

Light microscopic examination of immunohistochemically stained testis sections showed PCNA nuclear immunoreactivity which was strong and numerous in spermatogonia and moderate in primary spermatocytes of both control and T/D + MSCs groups (Figs. 7A&C). T/D group showed moderate immunoreactivity of few spermatogonia (Fig. 7B). Vimentin cytoplasmic immunoreactivity in Sertoli cells was distributed around the nuclei with apical extensions in a "spoke-like" pattern in control group (Fig. 7D). In T/D group, vimentin immunoreactivity was restricted around the nucleus only (Fig. 7E). T/D + MSCs showed increased vimentin expression with reestablishment of the spoke like pattern (Fig. 7F).

II-Morphometric and statistical results:

Serum testosterone levels (pg/ml):

Statistical analysis of the mean values of the serum testosterone levels showed a highly significant decrease in T/D group (II) as compared to both control group (I) and T/D with MSCs treatment group (III). By applying LSD (Least Significant Difference), for comparison between the groups, there was a highly significant decrease in serum testosterone level in group II when compared to the group (I) however, a non-significant decrease was detected between group (III) and control group (I) (Table1; Histogram1).

Height of the germinal epithelium (µm):

Statistical analysis of the mean values of the heights of the germinal epithelium lining the seminefrous tubules showed a highly significant decrease in the heights of the germinal epithelium in T/D group (II) as compared to both control group (I) and T/D with MSCs treatment group (III). By applying LSD, there was a highly significant decrease in the heights of the germinal epithelium in group II when compared to the control group. However, a non-significant decrease was detected between group III and group I (Table1; Histogram 2).

Area percentage (%) of PCNA immunoexpression:

Statistical analysis of the area % of PCNA immunoexpression showed a highly significant decrease in T/D group (II) as compared to both control group (I) and T/D with MSCs treatment group (III). By applying LSD there was a highly significant decrease in area % of PCNA immunoexpression in group II as compared to control group I however, a non-significant decrease was detected between group III and group I (Table1; Histogram 3).

Area % of vimentin immunoexpression:

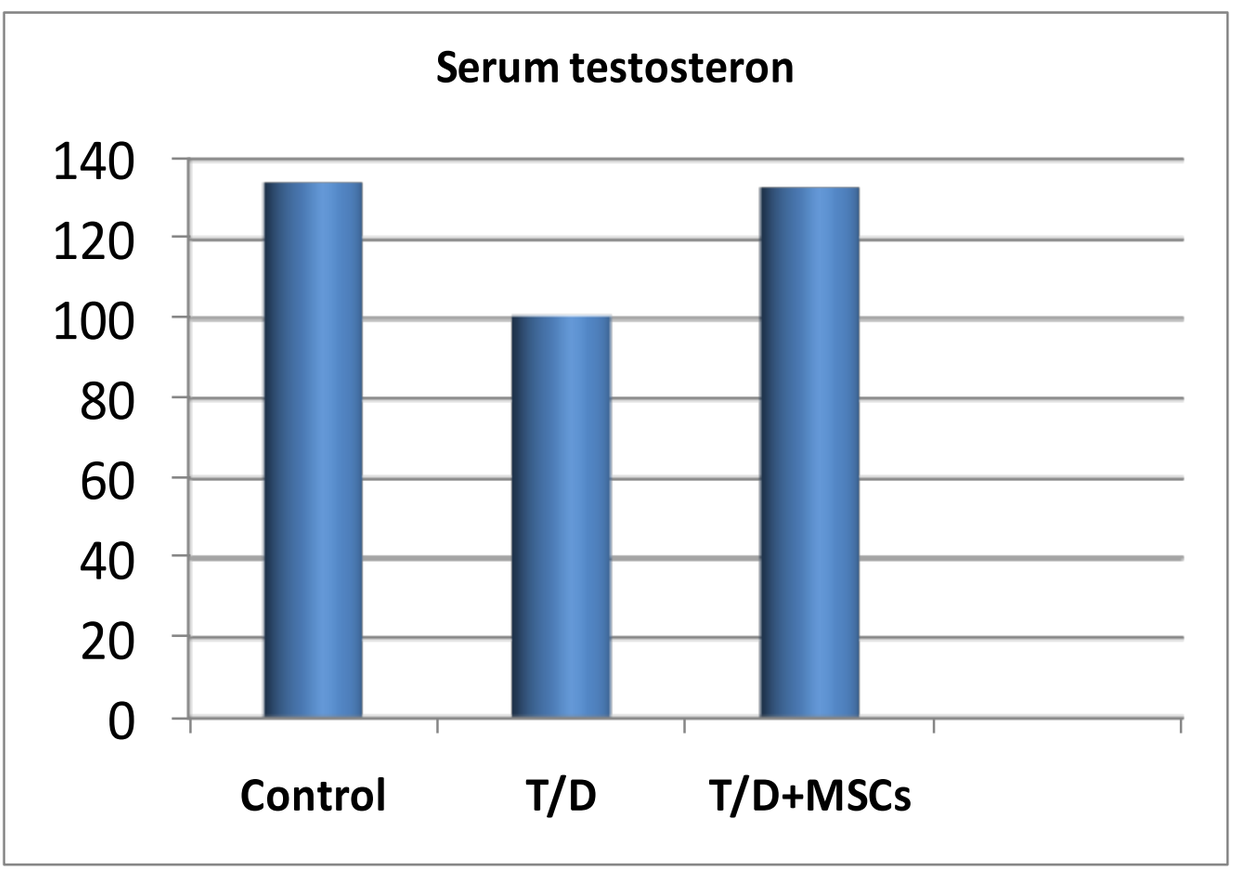
Statistical analysis of the area % of vimentin immunoexpression of showed a significant decrease in vimentin in T/D group (II) as compared to both control group (I) and T/D with MSCs treatment group (III). By applying LSD there was a significant decrease in area % of vimentin immunoexpression in group II as compared to control group I however a non-significant decrease was detected between group III and group I (Table1; Histogram 3).

Table 1: Statistical analysis of the mean values of serum testosterone level, height of the germinal epithelium, area % of PCNA immunoexpression and area % of vimentin immunoexpression among studied groups:

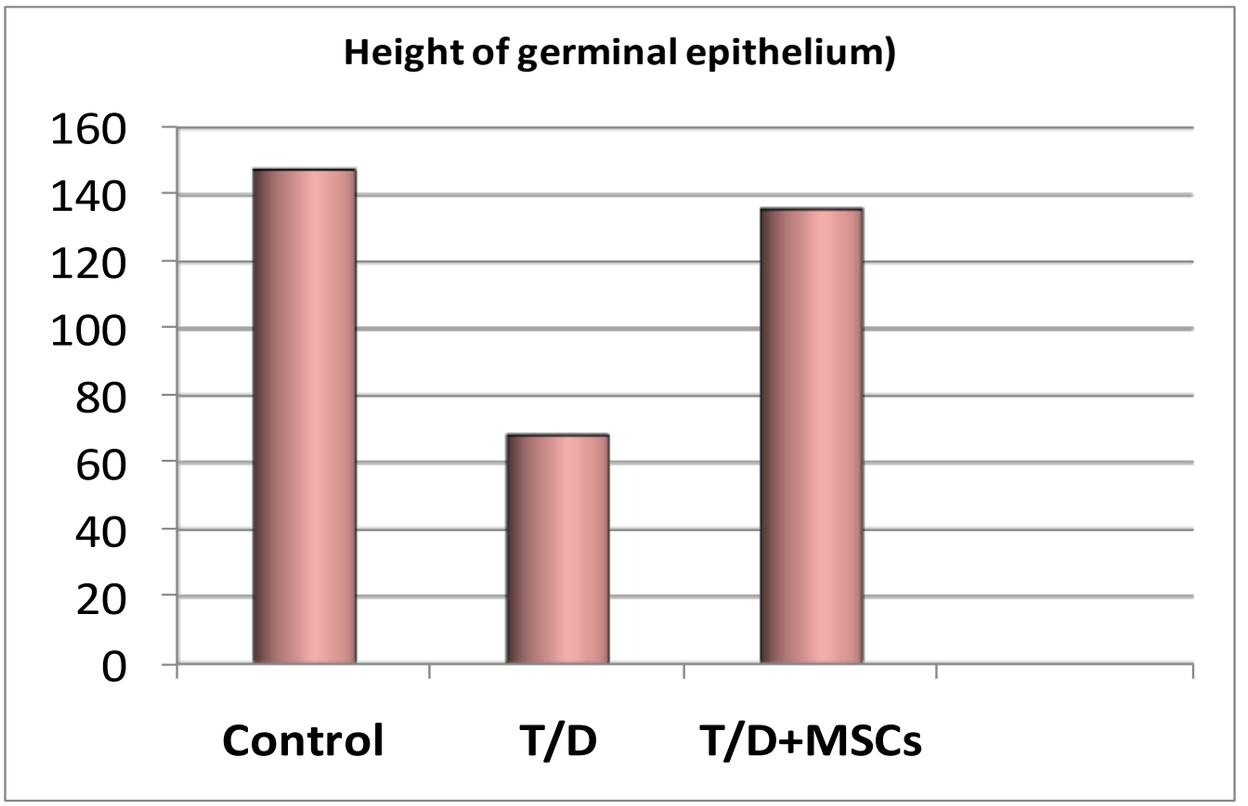
	Control Group (I) Mean±SD	T/D Group (II) Mean±SD	T/D+MSCs Group (III) Mean±SD	P
Serum testosterone level (pg/ ml)	134.2 ± 4.9	100.7 ± 4.5^{++}	132.96 ± 15.5^{NS}	0.000**
Height of the germinal epithelium (µm)	147.6 ± 15.5	68.2 ±16.1^{++}	135.8 ± 20.9^{NS}	0.000**
Area % of PCNA immunoexpression	33.4 ± 10.4	15.7 ± 6.9^{++}	25.9 ± 9.9^{NS}	0.000**
Area % of vimentin immunoexpression	40.03 ± 10.1	29.2 ± 7.3^{+}	38.1 ± 9.01^{NS}	0.018*

Highly significant difference when comparing T/D group with both control and T/D+ MSCs groups ($p<0.001$).
* Significant difference when comparing T/D group with both control and T/D+ MSCs groups ($p<0.05$).
++ Highly significant when comparing T/D group with control groups ($p<0.05$).
+ Significant difference when comparing T/D group with control group ($p<0.001$).
NS Non- significant difference when comparing T/D+ MSCs group with control group ($p>0.05$).

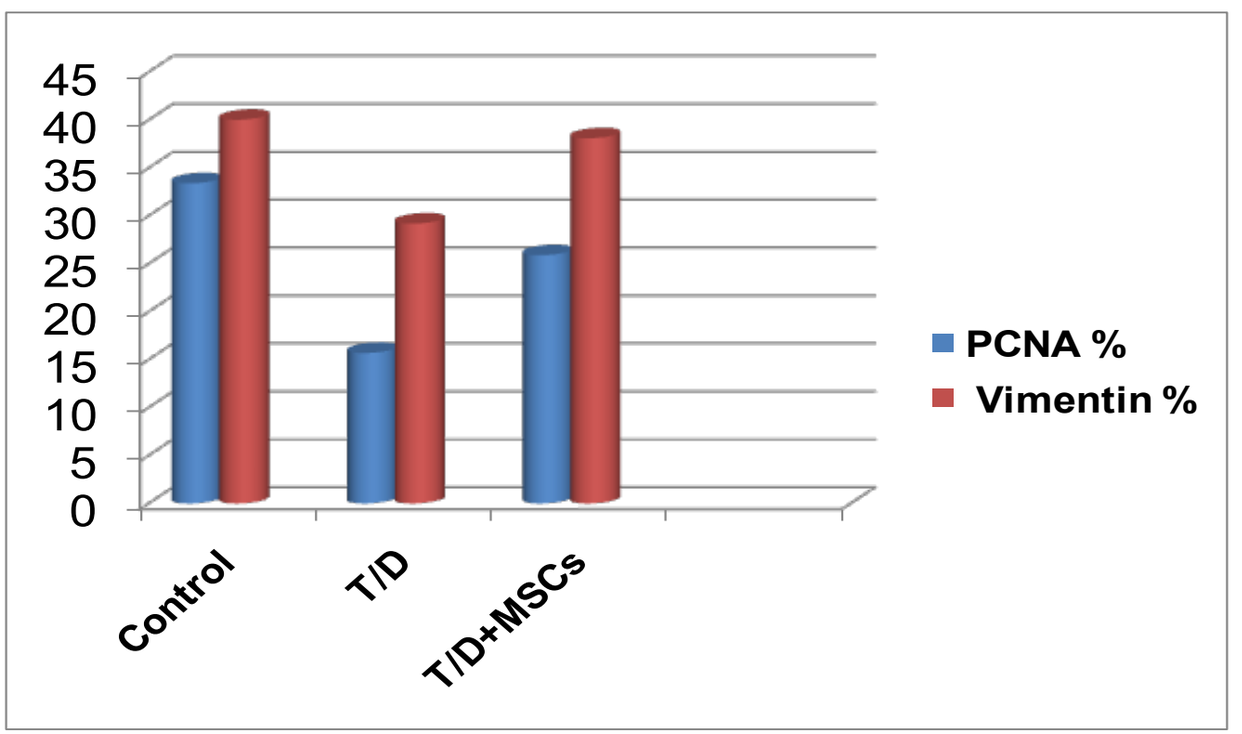
Histogram 1: The serum testosterone level among studied groups:



Histogram 2: The height of germinal epithelium among studied groups:



Histogram 3: The area percentage of PCNA nuclear immunoexpression and vimentin cytoplasmic immunoexpression among studied groups:



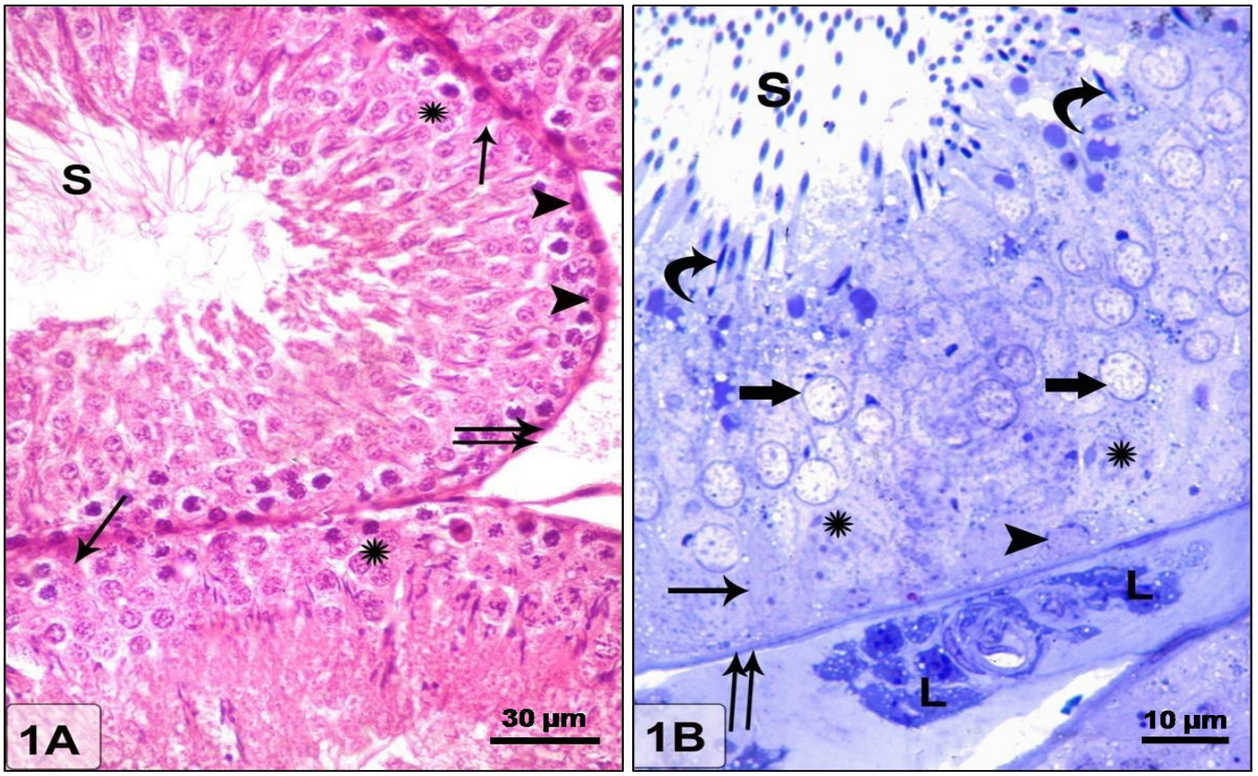


Fig. 1: Photomicrographs of testis sections from control group showing parts of seminiferous tubules with regular outline (double arrows) lined by Sertoli cells (thin arrows), spermatogonia (arrow heads), primary spermatocytes (*), early spermatids (thick arrows), late spermatids (curved arrows) and sperms (S). Interstitial Leydig cells (L) are also seen (A: H&E X400; B: Toluidine blue X 1000).

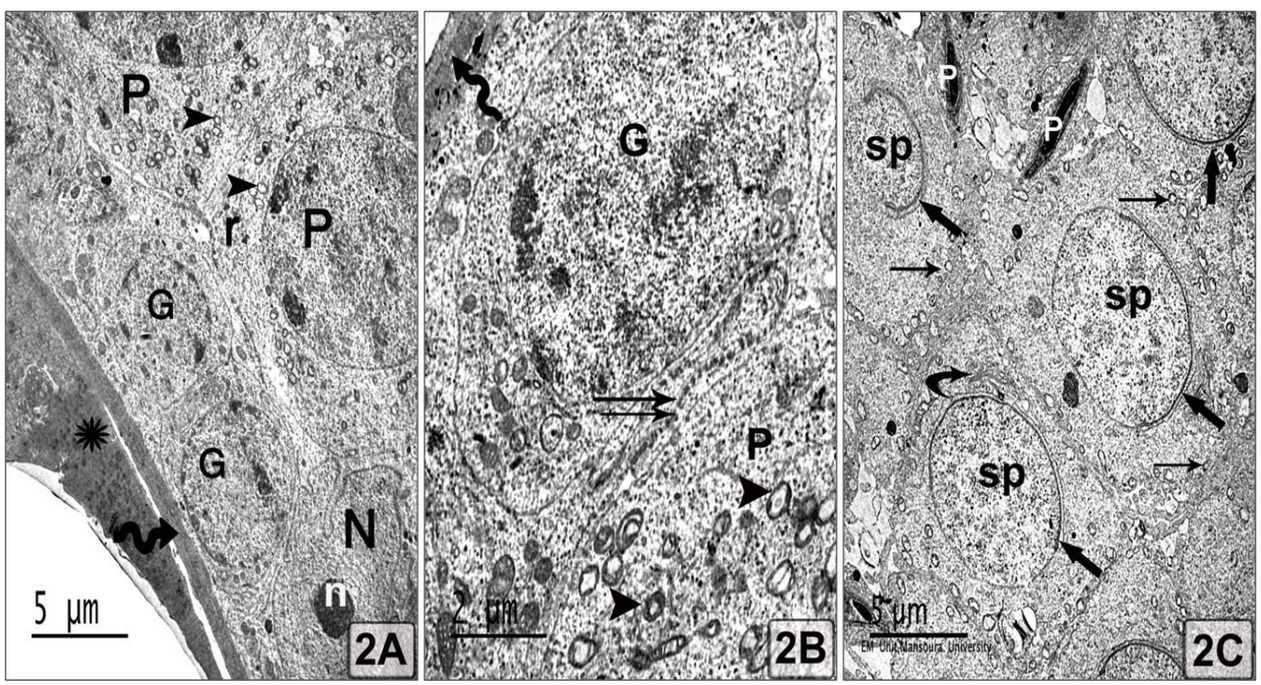


Fig. 2: Electronmicrographs of testis sections from control group showing (2A&B): Sertoli cell containing a pale euchromatic indented nucleus (N) and prominent nucleolus (n), spermatogonia (G) contains rounded nucleus with peripheral heterochromatin clumps and primary spermatocytes (black P) with rounded nuclei, mitochondria (arrowheads) and free ribosomes (r) and basement membrane (wavy arrow) surrounded by thin myoid cell (*) are also seen. (2B): Cross section in bundles of microfilaments of blood testis barrier (double arrows) is seen. (2C): Early spermatids with rounded euchromatic nuclei (sp), numerous peripherally arranged mitochondria (thin arrows), prominent Golgi cisternae (curved arrow) and acrosomal caps (thick arrows) on one side of the nucleus are observed. Late spermatids (white P) containing elongated condensed nuclei are also seen (Scale bar: A&C = 5µm; B = 2 µm).

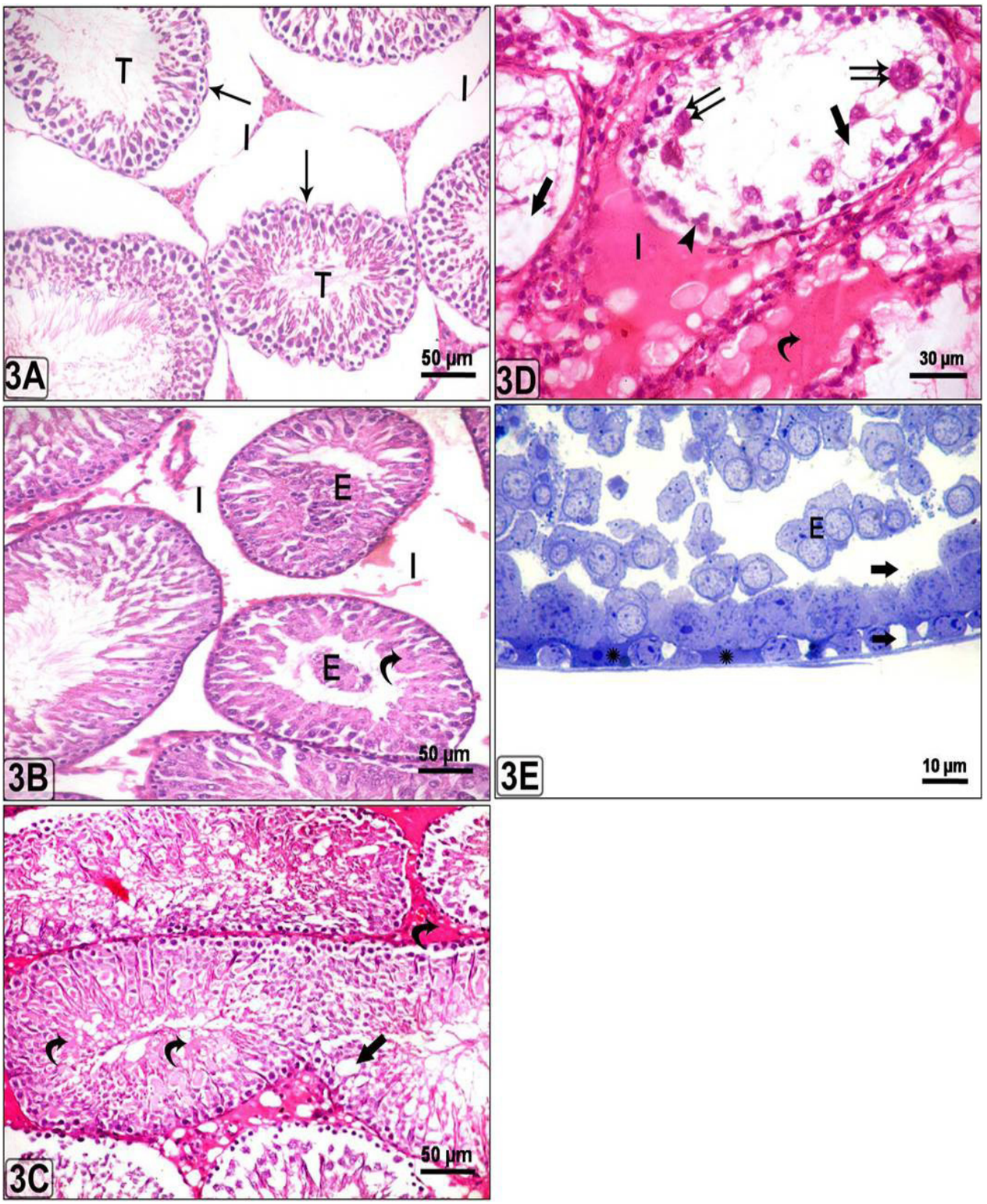


Fig.3: Photomicrographs of testis sections from T/D group showing (3A): Shrunken seminiferous tubules (T) with irregular outlines (thin arrows) separated by a wide interstitium (I). (3B-E): Exfoliated germ cells (E). (3C&D): Homogenous acidophilic material (curved arrows) within germinal epithelium and within the interstitium is observed. (3D&E): Areas of germ cells loss (thick arrows), giant cells (double arrows) and detached spermatogonia from underlying basement membrane (arrow head), Sertoli cells with darkly stained cytoplasm (*) are also seen (A-D: H&E, A-C X 200, D X400; E: Toluidine blue X 1000).

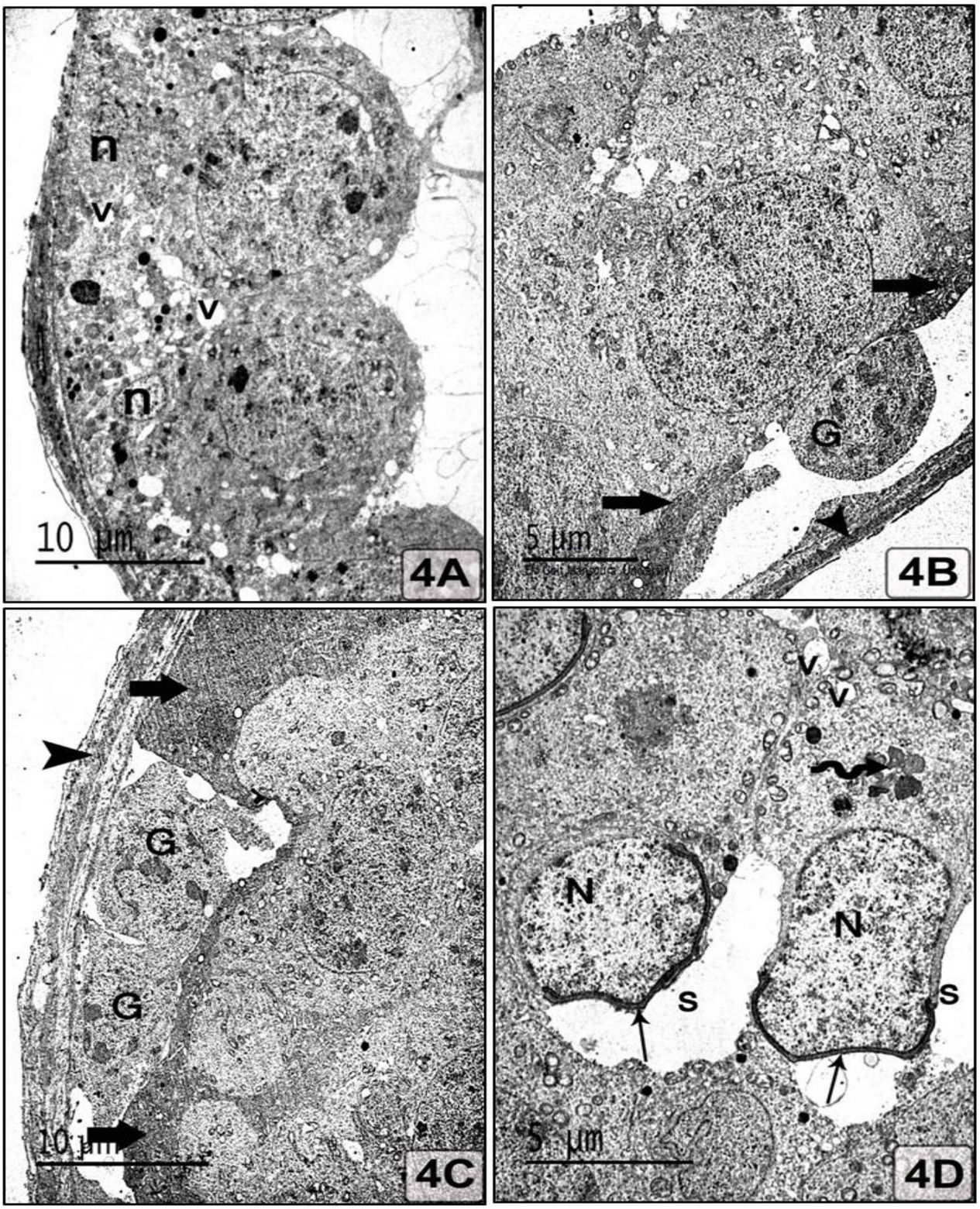


Fig. 4: Electronmicrographs of testis sections from T/D group showing (4A): Sertoli cells with small nuclei (n) and multiple vacuoles (v). (4B&C): Irregular spermatogonium (G) detached from both the basal lamina (arrow heads) and from electron dense cytoplasmic processes of Sertoli cell (thick arrows) with loss of blood testis barrier. (4D): Spermatids contain eccentric irregular nuclei (N), distorted acrosomal caps (thin arrows), vacuoles (v) and electron dense bodies (wavy arrow) with the presence of wide intercellular spaces (S) (Scale bar: A&B = 10µm; C = 5 µm).

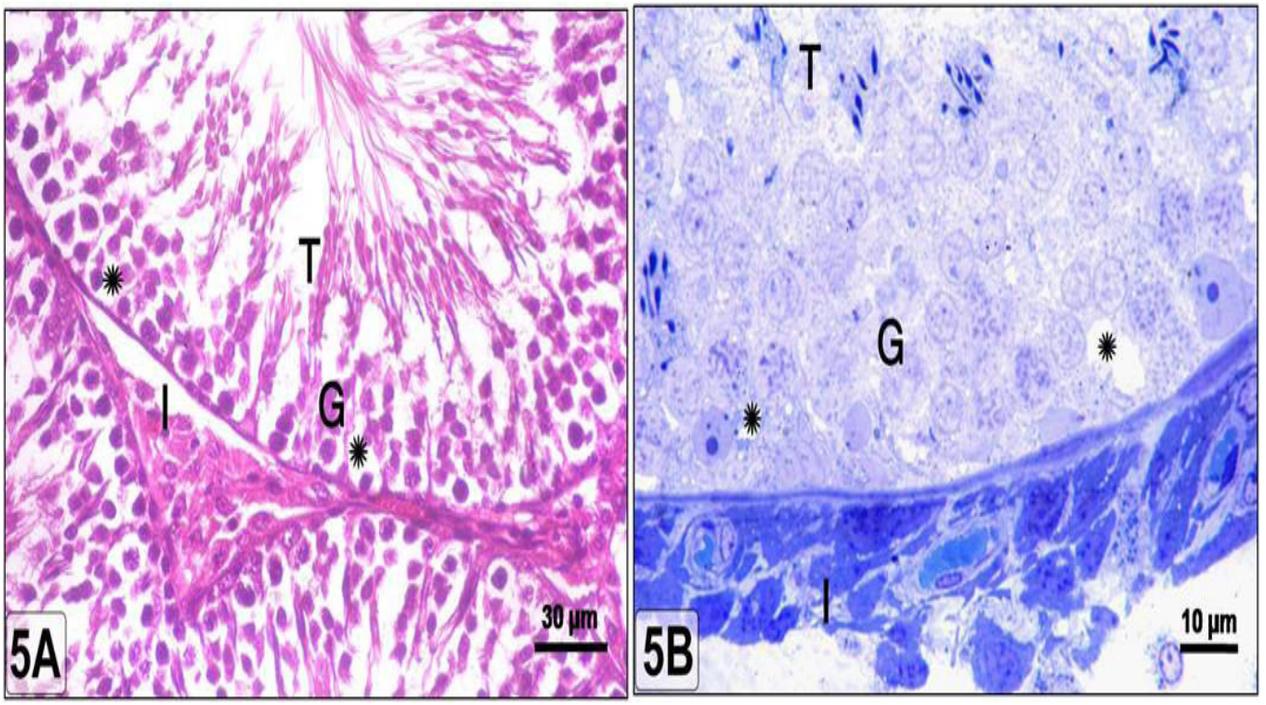


Fig. 5: Photomicrographs of testis sections from T/D + MSCs group showing apparently normal seminiferous tubules (T) lined by preserved germinal epithelium (G) with some intercellular spaces (*) and narrow interstitium (I) (A: H&E X400; B: Toluidine blue X 1000).

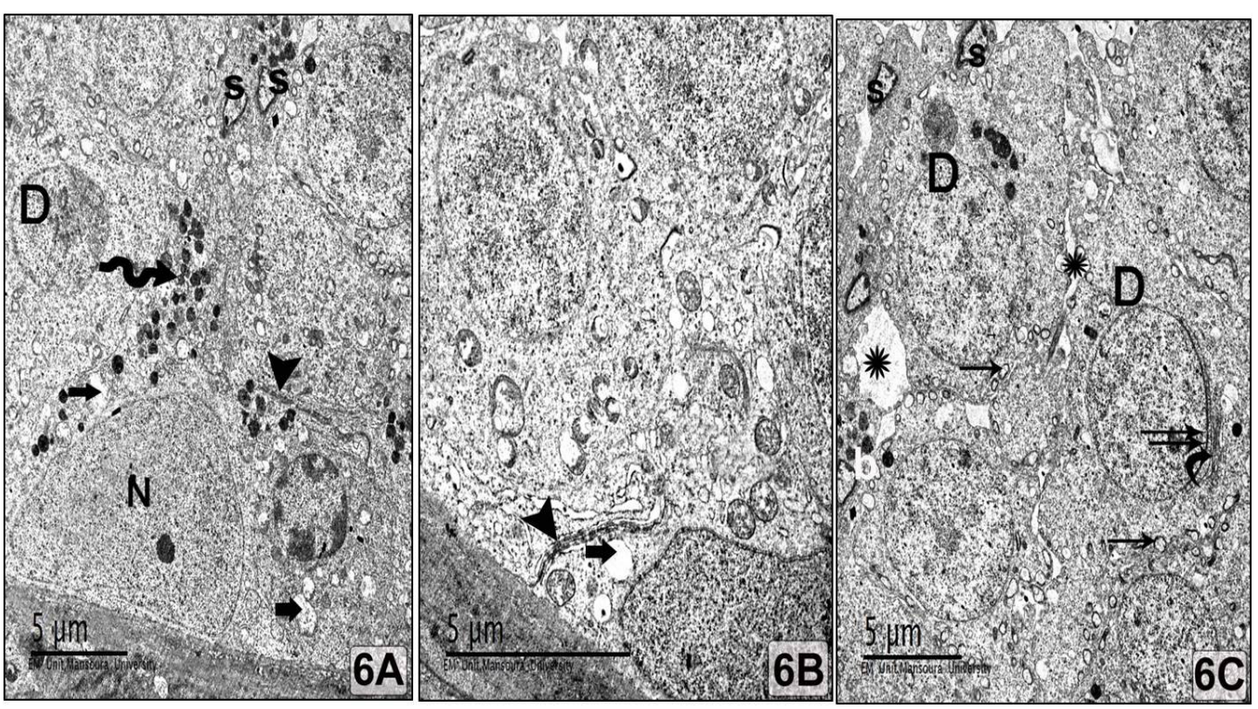


Fig. 6: Electronmicrographs of testis sections from T/D + MSCs group showing (6A&B): Sertoli cell containing euchromatic nucleus with prominent nucleolus (N) and cytoplasmic electron dense bodies (wavy arrows) and heads of some late spermatids (s) are impeded in Sertoli cell cytoplasm Some mitochondria with destructed cristae (thick arrows) are observed in the cytoplasm of spermatogonia and Sertoli cells. Preserved junction of blood testis barrier between Sertoli cells (arrow head) is noticed. (6C): Preserved early spermatids (D) with rounded euchromatic nuclei, peripherally arranged mitochondria (thin arrows), well-formed acrosomal cap (double arrows) and Golgi apparatus (curved arrow) are observed. Few intercellular spaces (*) and electron dense bodies (b) are also seen. (Scale bar = 5 µm).

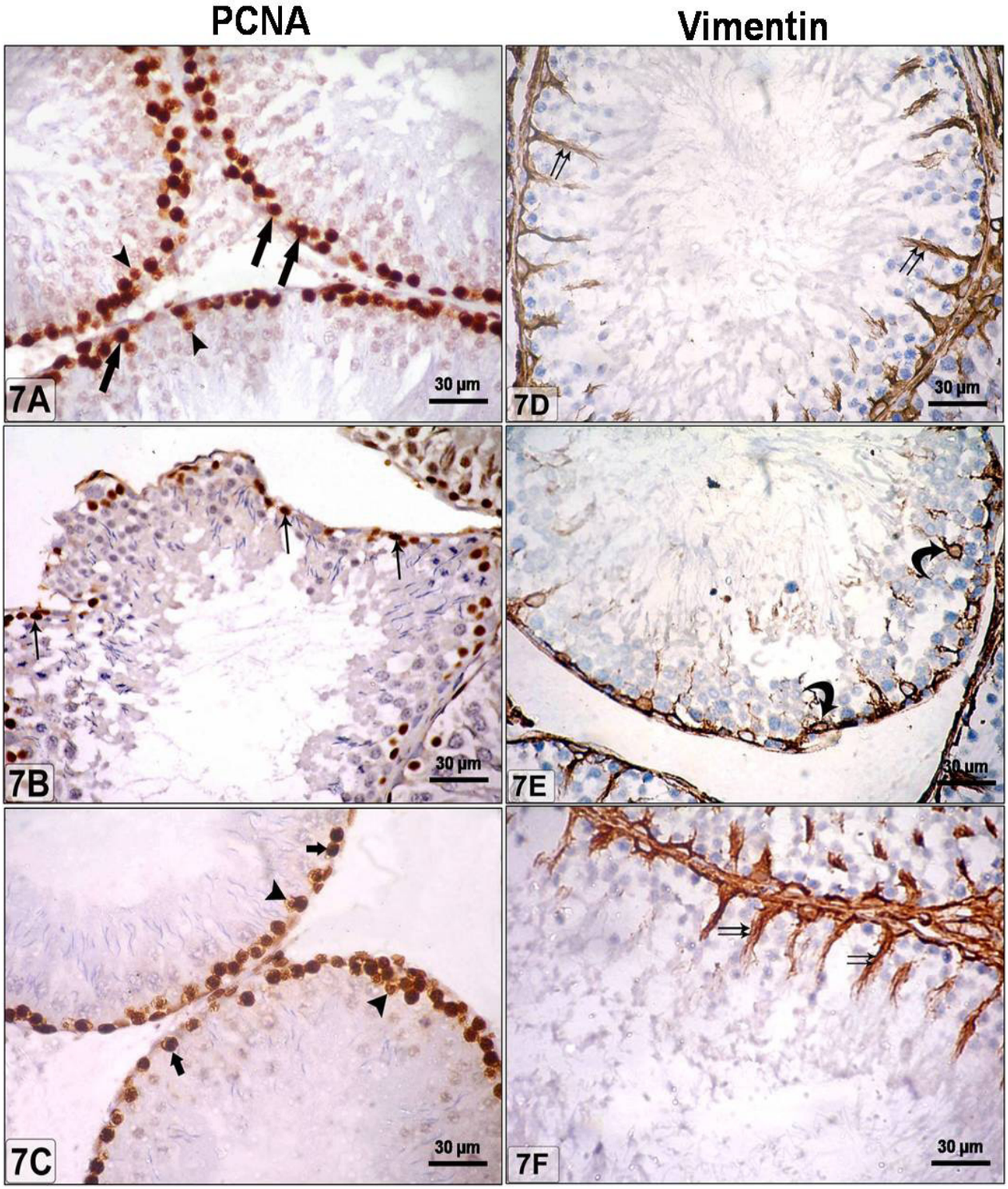


Fig. 7: Photomicrographs of testis sections showing PCNA nuclear immunoreactivity which is strong and present in numerous spermatogonia (thick arrows) and moderate in primary spermatocytes (arrow heads) of both control (7A) and T/D + MSCs (7C) groups. T/D group (7B) showing moderate immunoreactivity of few spermatogonia (thin arrows) Vimentin cytoplasmic immunoexpression in Sertoli cells distributed around the nucleus with apical extensions in a "spoke-like" pattern (double arrows) in control (7D) and T/D + MSCs (7F) groups. T/D group (7E) showing few Sertoli cells with vimentin immunoexpression restricted around the nucleus only (curved arrows). (A-C: Anti- PCNA X 400; D-F: Anti-Vimentin X 400).

DISCUSSION

Testicular torsion was recognized as an emergency. Thus, early diagnosis and appropriate treatment are fundamental to avoid irreversible testicular damage[17].

In the present work, serum testosterone of T/D group showed highly significant decrease in comparison to the control group that was accompanied with degenerative changes in seminefrous tubules. This is in accordance with Kurt and his colleagues[18]. That could be explained by Luo and his colleagues who reported that reactive oxygen species (ROS), produced due to ischemia reperfusion (I/R), can damage mitochondrial membranes and contribute to the inhibition of subsequent steroid production[19].

Light and electron microscopic examination of testis of T/D group showed degenerative changes of testicular parenchyma. These degenerative changes were confirmed by the observed significant reduction in the height of germinal epithelial lining seminiferous tubules in comparison with the control group. Similar findings were observed by previous investigators in rats after induction of testicular torsion for 30 minutes followed by detorsion with progression of testicular damage with the increase in reperfusion duration and they attributed that to the progressive oxidative stress [20]. Also, similar findings were previously detected after I/R[11]. These findings were in accordance with previous investigators[21]. Also, that could be explained according to Siu and his colleagues who reported that oxidative stress due to I/R led to appearance of cytoplasmic vacuolations in Sertoli cells with disruption of blood testis barrier[22]. Levine and his colleagues attributed the appearance of cytoplasmic vacuolations to the hydropic degeneration resulting from mitochondrial dysfunction and disruption of sodium pump with increased sodium influx and attraction of water[23].

Furthermore, during I/R, elevated ROS levels were reported to cause lipid peroxidation of cell membranes and DNA damage[24]. The same findings were observed by Creasy who attributed that to the high metabolic activity of seminiferous tubules beside their avascularity so, they depends on oxygen and nutrients transported from the interstitial blood vessels and hence, they are more susceptible to hypoxia with germ cells degeneration after testicular ischemic injury[25].

In the present work, some tubules showed lost and exfoliated germ cells with appearance of many vacuoles within germinal epithelium and within the interstitium. These sloughed germ cells were suggested to be spermatocytes and/or spermatids[20]. On the other hand, Creasy and his colleagues attributed the exfoliation of the germ cells, after phthalate esters toxicity, to loss of contact with the cytoplasmic processes of the surrounding Sertoli cell[25]. That could be supported by the observed disruption of blood testis barrier in the ultrastructure of the testis of T/D group in present work.

In the present study, the wide interstitium contained homogenous acidophilic material which was suggested to be due to an increase in vascular permeability[26]. On the other hand, many multinucleated giant cells were observed in the lumen of the seminiferous tubules. A similar observation was detected by previous investigators[20]. Testicular multinucleated giant cells were described as a degenerative process resulted from inability of tetraploid primary spermatocytes to complete the meiotic division or due to fusion of spermatids after the failure of intercellular bridges breakdown at early phases of spermiogenesis[25]. Moreover, Hild and his colleagues suggested that multinucleated giant cells could be due to aggregation of the sloughed degenerated spermatocytes and spermatids[27].

The results of present work showed that some sloughed early spermatids had eccentric irregular nuclei, many vacuoles, electron dense bodies and others had distorted acrosomal caps. Similar findings were observed by previous investigators who studied the histopathological changes caused by torsion/detorsion in wister rats[28].

The choice of PCNA as an immunohistochemical marker in the present study was according to D'Andrea and his colleagues who reported that the analysis of PCNA nuclear immunoexpression in the testis of rats could provide a quick, reliable, sensitive, and quantitative means to assess and detect early testicular toxicity[29]. In the control group of the present work, strong reaction was observed in most spermatogonia, and moderate reaction in other spermatogenic cells. The appearance of PCNA immunoexpression in meiotic spermatocytes, where no DNA duplication occurs, could be explained by the previously reported second function of PCNA in DNA excision repair during meiosis[30]. In the current study, statistical analysis of the mean values of area percentage of PCNA revealed a significant decrease in its immunoexpression in nuclei of spermatogonia in T/D group. Similar findings were observed by Kanter and his colleagues[31]. That might be attributed to the DNA damage by ROS released in testicular tissue after reperfusion[32].

In the current study, area percentage of vimentin immunoexpression was reduced in Sertoli cells of T/D group with disappearance of classical spoke-wheel-like structure of vimentin filaments from basal lamina to lumen. Similar results were previously described by Chen and his colleagues who suggested that

hypoxia condition may reduce vimentin filaments in the testis due to oxidative stress[33]. That may explain the observed degenerative changes in the present work with the detachment of spermatogenic cells from basal lamina and loss of blood testis barrier. That is in agreement with previous investigators who mentioned that, decreased vimentin filaments in Sertoli cell leads to detachment of spermatogenic cells from Sertoli cells with loss of the support and nutrition provided by Sertoli[34]. In addition, vimentin was reported to be one of cell junction proteins which is important for normal spermatogenesis and hence, the decreased density of vimentin could lead to blood-testis barrier disruption with spermatogenesis impairment[35].

In the present study, MSCs were injected intravenously to the experimental animals. The MSCs were chosen as they lack of surface antigens, so they don't activate the immune system besides their easy collection, rapid expansion and their less susceptibility to genetic mutations during in vitro passages[36]. In addition, MSCs have ability to migrate to the stroma of different tissues due to the expression of receptors by MSCs which are specific for cytokines released by damaged tissue[37,38].

In the present study, H&E stained sections of T/D with MSCs treatment group (T/D + MSCs) revealed apparently normal seminiferous tubules lined by well-formed germinal epithelium with less ultrastructure degenerative features. In agreement with the present study, Zahkook and his colleagues confirmed that MSCs had ability to differentiate into germ cells in vivo with restoration of testicular functions[39]. In consistence with these findings, previous investigators reported that the MSCs therapeutic effect, against torsion-induced germ cell injury, could be through reduction of intra-testicular oxidative stress, prevention of apoptosis and stimulation of testosterone secretion[40]. The effect of MSCs in spermatogenesis was previously suggested to be through changing the cellular microenvironment in which the germ cells grow[41].

In the current study, statistical analysis of the mean values of the area percentage of PCNA and vimentin immunoexpression showed non-significant reduction in the T/D + MSCs group beside reestablishment of the spoke wheel-like distribution of vimentin. Similarly, Helal and his colleagues observed increased immunoreactions for vimentin in the sarcoplasm of regenerating cardiac myocytes and vascular wall smooth muscles in rabbit injected with MSCs[42].

CONCLUSION

In conclusion, MSCs treatment could protect the spermatogenic and Sertoli cells from histopathological and immunohistochemical alterations, in the ipsilateral testis, after T/D in adult albino rats. Hence, MSCs therapy could be considered as a promising therapeutic tool to preserve spermatogenesis in cases of testicular T/D and should be more studied either on experimental animals or human.

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CONFLICT OF INTEREST

The authors have no conflicting financial interest.

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الملخص العربى

دور الخلايا الجذعية الوسيطة المشتقة من النخاع العظمى فى حماية الخلايا المولدة للنطاف وخلايا سيرتولى ضد التحورات النسيجية المحدثة بالألتواء و فك الألتواء فى الجرذان

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الخلفية: يعتبرالتواء الخصية حالة مرضية طارئة و شائعة تتسبب في موت خلايا الخصية و التأثير على الخصوبة.

الغرض من البحث: هدف هذا البحث لفحص التغيرات النسيجية في خصية الجرذ الابيض المتعرضة للالتواء/ فك الالتواء و توضيح دور حقن الخلايا الجذعية الوسيطة على هذه التغيرات.

المواد و الطرق: استخدم ٢٧ من ذكور الجرذان البيضاء و قسمت الى ثلاثة مجموعات متساوية: المجموعة الضابطة ، مجموعة الالتواء/ فك الالتواء بالاضافة لمجموعة الالتواء/ فك الالتواء مع العلاج بالخلايا الجذعية حقنا في وريد الذيل بعد العملية الجراحية.و بعد مرور عشرة اسابيع تم أخذ عينات من الخصية اليسرى واعدادها للفحص بالميكروسكوب الضوئي و الالكتروني.، وتم أخذ عينة من الدم لقياس نسبة التيستيستيرون.

النتائج: أظهر فحص الخصية لمجموعة الالتواء/ فك الالتواء وجود تغيرات تحللية فى الخلايا المولدة للنطاف وخلايا الدعم والتى كانت مصحوبة بانخفاض ذو دلالة احصائية فى مستوى هرمون التستوستيرون فى الدم مع انخفاض فى كل من ارتفاع النسيج الطلائى الجرثومى والنسبة المئوية للتعبير المناعى الهستوكيميائى لل PCNA و vimentin. و قد لوحظ نقص هذه التغيرات فى المجموعة التى تم حقنها بالخلايا الجذعية الوسيطة.

الاستنتاج: العلاج بالخلايا الجذعية الوسيطة يحمى الخلايا المولدة للنطاف وخلايا الدعم من التغيرات التحللية الناتجة عن التواء الخصية/ فك الالتواء في الجرذان البيضاء. و من ثم, من الممكن أن يعتبر العلاج بالخلايا الجذعية الوسيطة أداة علاجية واعدة للحفاظ على عملية الانطاف فى حالات التواء/فك الالتواء الخصية . ويجب ان يتم دراستها سواء على الحيوانات التجريبية أو على الانسان.