Effect of Silver Nanoparticles on Testes of Prepubertal Male Albino Rats and the Possible Protective Role of Vitamin E (Histological and Immunohistochemical Study)

Original Article

Khaled H. El-Mesalmy, Mohamed A. Shaheen, Noura H. Mekawy and Fatma Mosa

Department of Histology and Cell Biology, Faculty of Medicine, Zagazig University, Zagazig, Egypt

ABSTRACT

Background: Silver nanoparticles (AgNPs) are one of the most eminent and commonly used nanoparticles in customer everyday products for both adults and children.

Objective: This study objects to study the effect of prepubertal exposure of AgNPs on the histological structure of testis and the possible protective role of vitamin E.

Materials and Methods: Twenty three days old juvenile male albino rats were used and divided into 3 groups. Group I control: subdivided into two subgroups: subgroup Ia (negative control), Rats received no treatment and sacrified at postnatal day (PND) 23 and 58. Subgroup Ib (vehicle control group), further subdivided into two subgroup Ib1, Ib2. Group II: subdivided into 2 subgroups: Subgroup IIa; rats treated with AgNPs intraperitoneally daily at dose of (50 µg /kg/day) for 35 days from 23 PND to 58 PND and Subgroup IIb; treated with AgNPs and vitamin E (vit. E) orally by gavage at a dose (150 mg /kg / day) dissolved in corn oil from age 23-58 PND, Group III; follow up group, rats received AgNPs left without treatment till 90 PND.

Results: Testicular damage was also detected in AgNPs treated group as compared to the control group. Vit. E-treated subgroup and the follow up groups showed significant testicular improvement. A drastic decrease of testicular germinal epithelial height in AgNPs treated compared to control and vit. E treated rats. The expression of Bcl2 immunoreaction was also decreased in AgNPs-treated rats compared to the control and AgNPs and vit. E-treated groups.

Conclusion: AgNPs induced structural changes on male reproductive system that can be ameliorated by Vit. E supplementation and limiting the exposure to the products rich in these particles.

Received: 14 March 2020, **Accepted:** 16 June 2020

Key Words: Bcl2, silver nanoparticles, testis, vitamin E.

Corresponding Author: Noura H. Mekawy, MD, Department of Histology and Cell Biology, Faculty of Medicine, Zagazig University, Zagazig, Egypt, **Tel.**: +20 1125791103, **E-mail:** dr_nora184@yahoo.com

ISSN: 1110-0559, Vol. 44, No.2

INTRODUCTION

Silver nanoparticles (AgNPs) are used in many practical applications like medicine, industry, cosmetics, etc... These nanoparticles can be found in wound dressings, medical devices and food packs. Due to the widespread of AgNPs, humans get exposed to them everywhere in daily life by inhalation, orally, skin exposure, and by intravenous injection[1,2,3]. Some studies confirmed the toxicity of AgNPs to mammals while others have shown that they may induce genes associated with genetic injury[4]. So, it is mandatory to evaluate the safety of these particles on reproduction and fertility especially, in the prepubertal stage. Studies are rare in this developmental period and this matter requires more research[5].

Vitamin E (Vit. E) is a group of fat-soluble compounds. It is present in large quantities in vegetable oils and it has abundant essential roles in the body due to its antioxidant properties[6,7]. It is mainly originated in the membranes of cells and their organelles where it can do its maximal protective effect. It is also very important in defense against lipid peroxidation, thus protecting cell membranes from free radicals attack[8]. It is also well-known to be vital for spermatogenesis in mammalians[9].

MATERIALS AND METHODS

Chemicals

Silver nanoparticles

Silver nanopowder with a particle size less than 100 nm and a 99.9% trace metals basis purchased from Sigma-Aldrich Chemicals, Cairo, Egypt. Chemical Abstract Service registration Number (CAS No) is (7440-22-4). Polyvinylpyrrolidone (PVP) was used as dispersant (Figure 1).

 DOI: 10.21608/ejh.2020.25855.1261

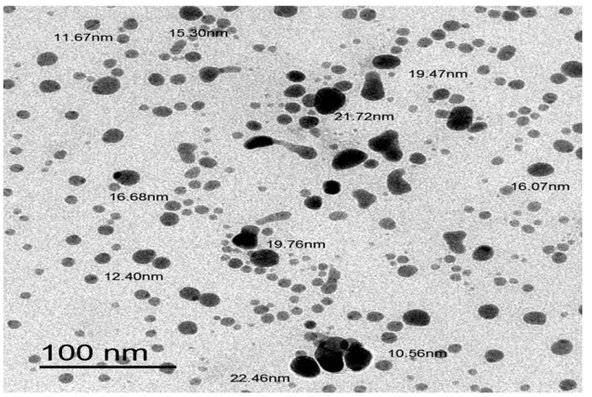

Fig. I: An electron micrograph showing average diameters of AgNPs.

Vit. E

400 mg oil capsules, from Sigma-Aldrich Chemicals, Cairo, Egypt.

Corn oil

solvent for Vit. E.

Animals and experimental design

56 healthy juvenile male albino rats (23 days old) with average weight 70-90 gm were used in this experiment, housed at animal house of Faculty of Medicine, Zagazig University at room temperature, fed standard balanced regimen and allowed water ad-libitum. The rats were permitted to adjust to their new surrounding environment for 5 days. All experimental procedures were done in agreement to guidelines of Medical Researsh Ethics Committee of Zagazig University (the protocol approval number: 4663). This committee follows the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Rats were randomly divided into three groups; Group I (control group): contained thirty two rats further subdivided into two subgroups:

Subgroup Ia (negative control group): Contained 16 rats; this group received no treatment to measure the basic parameters. Eight rats were sacrificed at age 23 PND. The remainders were sacrificed at age 58 PND[10].

Subgroup Ib (vehicle control group): included 16 rats equally subdivided into two subgroups:

Subgroup Ib1: Rats received 1 ml saline daily intraperitoneally from age 23 to 58 PND. Saline solution is the solvent for silver nanoparticles[10].

Subgroup Ib2: Rats received corn oil 0.5 ml /day orally by gavage from age 23-58 PND. Corn oil is the solvent for vit. E[11].

Group II: Included 16 rats, further subdivided into 2 equal subgroups:

Subgroup IIa (AgNPs treated subgroup): Rats received AgNPs intraperitoneally at dose of (50 µg /kg/day)[12] dissolved in saline solution daily from age 23 to 58 PND[10].

Subgroup IIb (AgNPs and vit. E treated subgroup): Rats received AgNPs as group IIa and vit. E orally by gavage at a dose (150 mg /kg /day) dissolved in corn oil from age 23-58 PND[10,11].

Group III (follow up group): Included 8 rats, they received intraperitoneal injection of AgNPs as group IIa then left without treatment for 32 days till they reached PND 90[12].

At the end of the experiment, all rats were anaesthetized with 50 mg/kg body weight of sodium phenobarbital through intra-peritoneal injection[13]. Blood samples were taken for measuring serum testosterone level. Then, the testicles specimens were excised and processed for histological study (light microscopic and electron microscopic examination).

Biochemical study (Measurement of serum testosterone level): serum was obtained by centrifugation (1300 x g for 20 minutes at 4°C) and examined in department of Clinical Pathology, Faculty of medicine- Zagazig University using commercial Randox kits for measuring serum testosterone level by enzyme-linked immunosorbent assay (ELISA). The results were collected and statistically analyzed[14].

Histological and Immunohistochemical Studies

Histological studies

For light microscopy: Testes were immersed in Bouin fixative for 24 hours, processed and stained with Hematoxylin and Eosin[15].

Immunohistochemical study

Paraffin-prepared sections were stained using the avidin–biotin peroxidase system for the detection of Bcl2 as antiapoptotic protein (CAS No. 85878, Sigma-Aldrich, Steinheim, Germany) using primary antibody (rabbit polyclonal antibody) (product No. LS-C78828; Life span Biosciences, Inc. seattle, WA, USA). Then, they were incubated for two hours with secondary anti-rabbit antibody (Zymed laboratories). Staining was completed by using diamiobenzidine (DAB) according to[16].

For electron microscope preparation; testicular specimens were fixed in fresh 3% glutaraldehyde (pH. 7.4), post fixed in 1% osmium tetroxide in the same buffer at 4°C, dehydrated and embedded in epoxy resin. Then, semi-thin for toluidine blue stain was obtained. Ultrathin sections were obtained and processed for electron microscope examination using a JEOL JEM 2100 electron microscope (Jeol Ltd, Tokyo, Japan) in Electron Microscope Research Laboratory of Faculty of Agriculture, El Mansoura University, Egypt[17,18].

Morphometric study

Sections stained with H&E and immunostained for Bcl2 were morphometrically analyzed using the Fiji Image J (1.51n, NIH, USA). All the parameters were measured for the randomly chosen five fields per section in total five sections from each group.

Statistical analysis

Performed using SPSS software (version 16.0, Chicago, USA) via using one-way analysis of variance (one-way ANOVA). The attained data from biochemical analysis (serum testosterone), morphometrical analysis (germinal epithelial height, and Bcl2 immunoreactions) were presented as mean±SD and studied using (ANOVA) followed by the post hoc test for multiple comparisons between different groups. The probability values (*P*) less than 0.05 were considered statistically significant and highly statistically significant when *P value* <0.001and non-significant when *P value* >0.05[19].

RESULTS

General observations: During the study period, rats of AgNPs treated group exhibited fatigue, decreased food consumption, reduced locomotor activity. In AgNPs+vit. E and follow up groups, there was a decrease in the incidence and severity of the previous signs. No treatment-associated mortality was observed in all groups.

Biochemical analysis

Hormonal assay of serum testosterone level

The level of serum testosterone in 23 days control rats was significantly less than the corresponding values in the other groups. However, testosterone values in all other groups were not significant (Table.1).

Histological results

Group I (Control group): subgroup Ia (23 days old)

Examination of H&E stained-sections of testis of 23 days control rats revealed parenchyma of testis was formed of small rounded seminiferous tubules surrounded by tunica albuginea. Seminiferous tubules were lined by stratified germinal epithelium resting on a regular basement membrane. Most of them had narrow lumina. Clusters of Leydig cells were found in the narrow interstitium (Figures 2a, 2b). Immuno-histochemical sections stained with Bcl2 protein exhibited positive cytoplasmic immunoreaction in most cells of the germinal epithelium and in the Leydig cells (Figure 2c). Toluidine blue stained semithin sections showed that epithelium of tubules lined with several types of spermatogenic cells. These tubules were surrounded by myoid cells. The interstitial tissue contained small groups of Leydig cells and blood capillaries (Figure 2d).

Examination of ultrathin sections of previous group showed primary spermatocytes with a large heterochromatic spherical nucleus. Their cytoplasm contained mitochondria and free ribosomes. Sertoli cells had euchromatic nuclei with prominent nucleoli, and many mitochondria. Early spermatids appeared with large rounded nuclei and fine chromatin. Their cytoplasm contained peripherally arranged mitochondria (Figures 3a, 3b). Leydig cells nuclei were heterochromatic and ovoid in shape. Their cytoplasm contained smooth endoplasmic reticulum, mitochondria and lysosomes (Figure 3c).

Examination of 58 days control rats of subgroups Ia and Ib revealed nearly similar histological results; consequently, only results of the control subgroup Ia were presented.

H&E stained sections revealed normal testicular parenchyma with packed larger seminiferous tubules compared to the control prepupertal group surrounded by regular connective tissue capsule. Tubules were lined by stratified germinal epithelium. Interstitium exhibited groups of Leydig cells with oval nuclei and acidophilic cytoplasm and blood capillaries (Figures 4a, 4b). AgNPs treated subgroup there was shrunken disorganized seminiferous tubules with marked reduction in epithelium thickness with few spermatozoa.

Marked separation between germinal epithelial cells was also seen. Germ cells were few and many cells exhibited deeply stained nuclei. Interstitium had congested blood vessels with homogenous vacuolated eosinophilic material (Figures 4c. 4d). In AgNPs and Vit. E-treated subgroup the covering capsule is regular, most of seminiferous tubules had nearly normal architecture and their wall was formed of nearly normal arranged germinal epithelium. Most of their lumina contained aggregation of sperms. There were near to normal width of interstitium in-between the tubules with little homogenous vacuolated eosinophilic material (Figures 4e, 4f).

In the follow up group, tubules were covered by slightly irregular capsule. some semineferous tubules apparently resumed their normal general structure. They had moderately arranged germinal epithelium with little intercellular spaces. They were separated by wide interstitium which contained leydig cells and eosinophilic vacuolated exudate (Figure 4g, 4h).

Immuno-histochemical stained sections of 58 days control rats for Bcl2 protein displayed positive cytoplasmic immunoreaction in most cells of the germinal epithelium and Leydig cells (Figure 5a).

AgNPs-treated subgroup there was weak cytoplasmic immunoreaction in few cells of germinal epithelium and Leydig cells (Figure 5b). Interestingly, AgNPs and Vit. E-treated rats showed nearly normal cytoplasmic immunoreaction in the cells of germinal epithelium and Leydig cells (Figure 5c). Moreover, examination of follow up group revealed moderate cytoplasmic immunoreaction in the cells of germinal epithelium and Leydig cells (Figure 5d).

Toluidine blue stained semithin sections of 58 days control group showed that seminiferous tubules were lined by different types of spermatogenic cells. Spermatids occupied several layers at the adluminal compartement. The sperm heads appeared penetrating a large depth of adluminal compartement. The interstitium exhibited clusters of Leydig cells (Figure 6a). In AgNPs-treated subgroup, there were separation between germinal epithelial cells and many vacuoles. They were lying on irregular basement membrane. Wide interstitium with a thick wall blood vessel was also

observed (Figure 6b). However, AgNPs and Vit. E-treated subgroup showed nearly normal seminiferous tubules with apparently normal lining epithelium enclosed by regular basement membrane with few spaces between germinal epithelial cells (Figure 6c).

Examination of follow up group revealed seminiferous tubules with moderately organized epithelium enclosed by slightly irregular basement membrane. Many spaces and vacuolations were seen between germinal epithelial cells (Figure 6d).

Ultrastructurally, 58 days control rats revealed that the seminiferous tubules were lined by spermatogonia that had thin rim of cytoplasm and rounded nuclei with marginated heterochromatin. Primary spermatocytes had large rounded nuclei and electron dense heterochromatin. Spermatids appeared with large ovoid euchromatic nuclei. Sertoli cells contained euchromatic nucleus and large number of mitochondria and lysosomes. The cytoplasm had numerous peripherally situated mitochondria. More developed spermatid appeared with ovoid euochromatic nucleus with acrosomal cap with well-formed acrosomal vesicle (Figures 7a, 7b, 7c). Middle pieces of sperm tails had central axoneme, surrounded by nine thick course dense fibers, mitochondrial sheaths and cell membranes (Figure 7d). Interstitial cell of Leydig had spherical nuclei and the cytoplasm contained smooth endoplasmic reticulum and mitochondria and significant amount of lipid droplets (Figure 7e).

While, AgNPs-treated subgroup exhibited partial separation of spermatogonia from basement membrane and from surrounding cells. They revealed heterochromatic nucleus and cytoplasmic vacuoles. Primary spermatocytes had irregular shaped nuclei and their cytoplasm contained disorganized mitochondria and cytoplasmic vacuoles. Sertoli cells had electron dense nuclei and cytoplasmic vacuoles. They had mitochondria with disrupted cristae and lysosomes (Figures 8a, 8b, 8c). Late spermatids appeared with eccentric nuclei and disarranged mitochondria beneath irregular cell membranes. Many vacuoles and intercellular spaces were also observed (Figures 8d, 8e). Cross sections of middle piece of mature sperms revealed swollen mitochondrial sheath and irregular cell membrane. Some pieces showed cytoplamic vacuoles (Figure 8f). Leydig cells had irregular nuclei with peripheral heterochromatin and widened nuclear envelope (Figure 8g). On the other hand, examination of AgNPs and vit. E-treated subgroup revealed spermatogonia with heterochromatic nuclei, Primary spermatocytes had large rounded nuclei with scattered heterochromatin masses. Sertoli cells appeared with large euchromatic nuclei. Spermatids displayed rounded euchromatic nuclei and peripherally situated mitochondria. Little spaces were also found in-between cells (Figures 9a, 9b). Cross sections in the tails of sperms showed the middle, principal and end pieces which had normal structure (Figure 9c). Leydig cells were seen with oval regular euchromatic nuclei with peripheral heterochromatin and their cytoplasm contained lipid droplets (Figure 9d). In the follow up group, some cells had euchromatic nuclei with scattered heterochromatin particles. However, other cells had shrunken nuclei with irregular nuclear envelopes. Early spermatids appeared with rounded euchromatic nuclei and mitochondria. Paranuclear mitochondria also appeared in the cytoplasm. Sertoli cells also appeared separated from basement membrane (Figures 10a, 10b). Cross sections of tails of sperms revealed that some pieces had normal structure with well-organized axoneme and fibrous sheath. However, some pieces showed ruptured cell membrane and many vacuoles (Figure 10c). Leydig cells showed oval euchromatic nuclei with irregular nuclear envelope and peripheral heterochromatin particles (Figure 10d).

Morphometrical results

The epithelial height of seminiferous tubule in random fields showed a significant decrease in AgNPs-treated group compared to the control and AgNPs+Vit. E-treated groups. However, they were significantly increased in AgNPs+Vit. E-treated group compared to the follow up group (Table 2).

The Optical density of immunoreaction to Bcl2 showed a drastic decrease in AgNPs group compared to the control and AgNPs+Vit. E-treated groups. However, there were dramatically increased in AgNPs+Vit. E-treated group compared to the follow up group (Table 3).

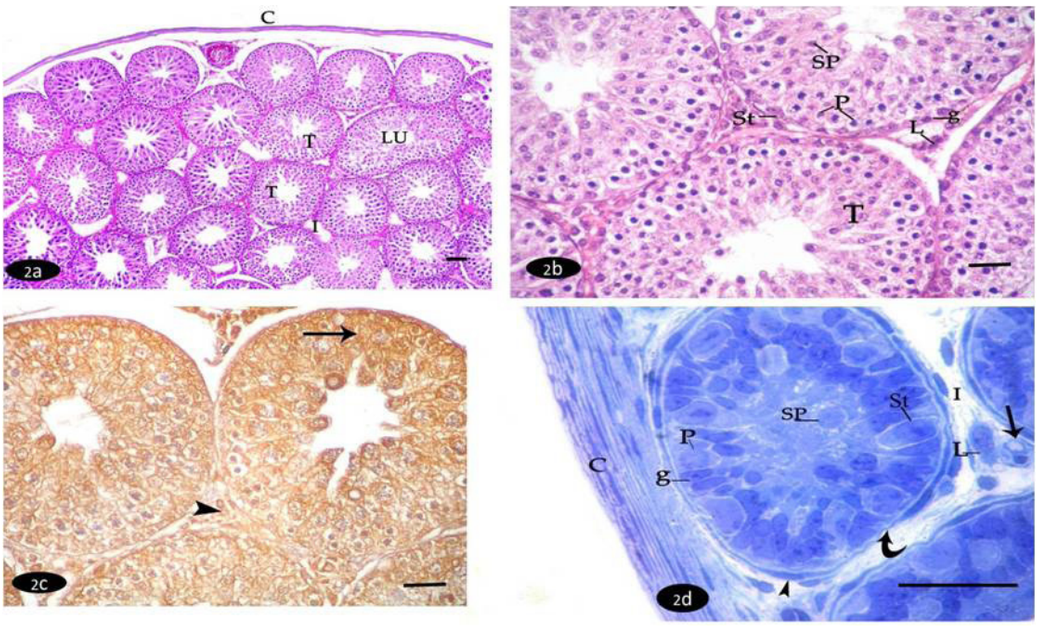

Fig. 2: a): A photomicrograph of 23 days control rats showing small rounded packed seminiferous tubules (T) with narrow lumina (LU), separated by narrow interstitium (I). They are surrounded by regular connective tissue capsule (C) (H&E X100 scale bar 30µm). b): Seminiferous tubules (T) are lined by spermatogonia (g), primary spermatocytes (P), spermatids (SP) and Sertoli cells (St). Interstitium contains clusters of leydig cells (L). (H&E X400 scale bar 30µm). c): Immunohistochemical examination of the same group showing strong positive cytoplasmic immunoreactions to Bcl2 in most cells of germinal epithelium (arrow) and in the Leydig cells (arrow head). (Immunoperoxidase for Bcl2 protein x400 scale bar 30µm). d): Toludine blue semithin section showing seminiferous tubules surrounded by regular capsule (C). They are lined by spermatogonia (g), primary spermatocytes (P) and spermatids (SP). Sertoli cell (St). They are resting on regular basement membrane (curved arrow) ensheathed by flat myoid cells (arrow head). Interstitium shows clusters of Leydig cells (L) and blood capillaries (arrow) (Toluidine blue x 1000 scale bar 30µm).

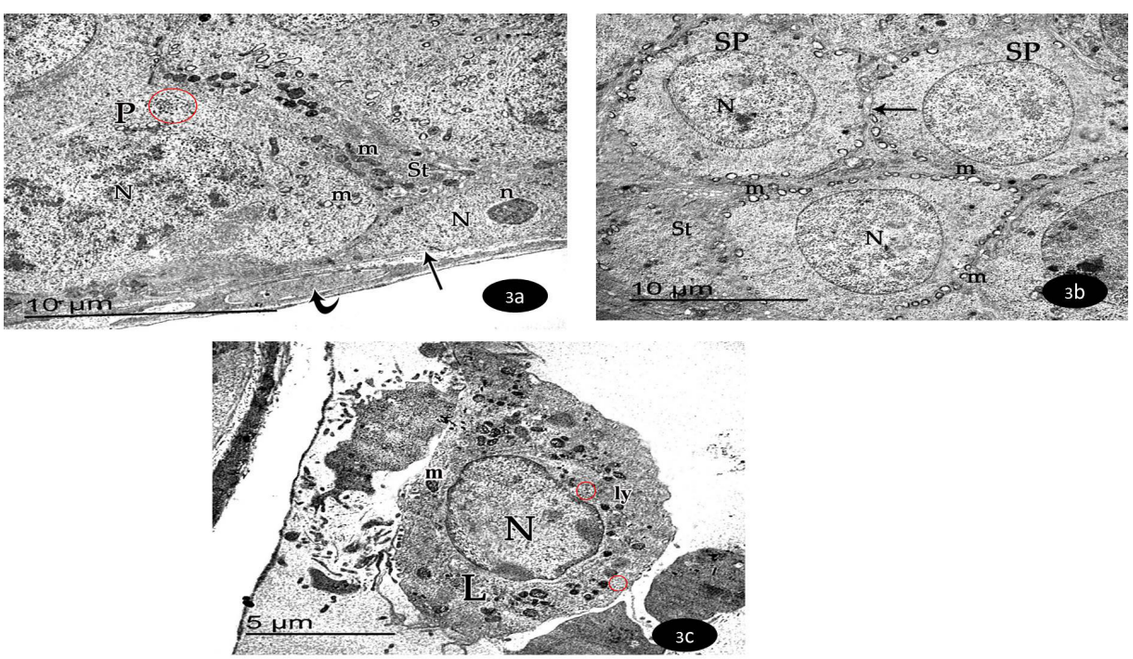

Fig. 3: a): An electron micrograph from 23 days control rat showing a primary spermatocyte (P) and Sertoli cell (St) resting on regular basement membrane (arrow) ensheathed by myoid cell (curved arrow). Primary spermatocyte has large spherical nucleus (N). Its cytoplasm contains mitochondria (m) and free ribosomes (circle). Sertoli cell has euchromatic nucleus (N) and prominent nucleolus (n) with many mitochondria (m) X6700 b): Early spermatids (SP) with large rounded nuclei (N), their cytoplasm contains peripherally arranged mitochondria (m). c): Leydig cell (L) with ovoid nucleus (N). Cytoplasm contains cisternae of smooth endoplasmic reticulum (circle) mitochondria (m) and lysosomes (ly) X8400.

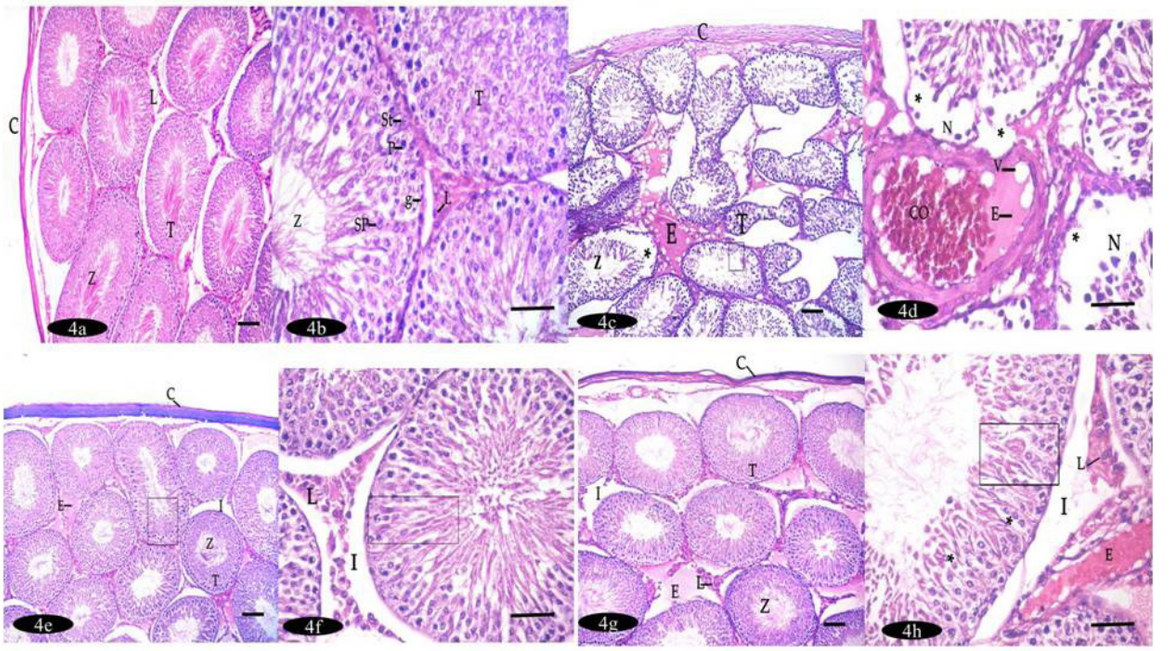

Fig. 4: a): A photomicrograph of a section in the testis of 58 days control rats showing testicular parenchyma consists of packed seminiferous tubules (T) and separated by narrow interstitium containing clusters of leydig cells (L). They are covered by regular connective tissue capsule (C). Lumena are patent and contain clumps of spermatozoa (Z) (H&E X100scale bar 30µm). b): Seminiferous tubules (T) lined by spermatogonia (g), primary spermatocytes (P), spermatids (SP) and sertoli cells (St). The lumen of the tubule contains spermatozoa (Z) and the interstitium contains Leydig cells (L) (H&E X400scale bar 30µm). c): AgNPs treated subgroup many shrunken disorganized seminiferous tubules (T) with marked reduction in the thickness of their linning germinal epithelium (rectangle). Other tubules reveal reduction in the number of spermatozoa (Z). Separation and spaces (star) between germinal epithelial cells are also noticed. The interstitium in-between is wide and contains vacuolated eosinophilic material (E) (H&E X100scale bar 30µm). d): Marked separation (star) between the germinal epithelial cells. Many germinal cells exhibited deeply stained nuclei (N). A congested blood vessel (CO) with homogenous vacuolated (V) eosinophilic material (E) in the interstitium (H&E X400scale bar 30µm). e): AgNPs + vit. E treated subgroup most of seminiferous tubules (T) having nearly regular contour, normally arranged germinal epithelium (rectangle) and most of their lumina contain aggregations of spermatozoa (Z). There is apparently normal width of interstitium in-between the tubules (I) with little homogenous vacuolated esinophilic material (E). They are covered by regular capsule (C) (H&E X100scale bar 30µm). f): Nearly normal lining germinal epithelium (rectangle). Interstitium (I) is slightly wide with clusters of Ledyig cells (L) (H&E X400scale bar 30µm). g): Examination of follow up group showing some semineferous tubules (T) apparently regain its normal shape with apparently normal arranged germinal epithelium, sperms in their lumina (Z). They are separated by wide interstitium (I) which contain leydig cells (L) and eosinophilic vauolated exudate (E).Tubules are covered by slightly irregular capsule (c) (H&E X100scale bar 30µm) h): moderately organized germinal epithelium (rectangle). Little intercellular spaces (star) are noticed. Interstitium (I) is still wide with some leydig cells (L) and eosinophilic material (E) (H&E X400scale bar 30µm).

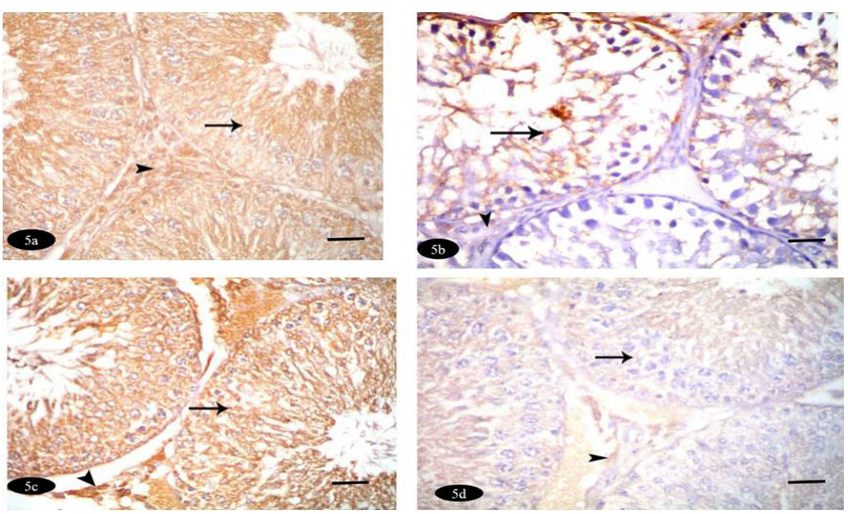

Fig. 5: a): A photomicrograph of a section in the testis of 58 days control group showing strong positive cytoplasmic immunoreaction to Bcl2 in most cells of germinal epithelium (arrow) and in Leydig cells (arrow head). b): AgNPs treated subgroup reveals weak positive cytoplasmic immunoreaction to Bcl2 in few cells of germinal epithelium (arrow) and in the Leydig cells (arrow head). c): AgNPs and vit E-treated subgroup stained sections showing strong positive cytoplasmic immunereaction to Bcl2 in germinal epithelium (arrow) and in Leydig cells (arrow head) d): In follow up group there are moderate positive cytoplasmic immunereaction to Bcl2 in germinal epithelium (arrow) and Leydig cells (arrow head) (Immunoperoxidase for Bcl2 protein x 400 scale bar 30µm)

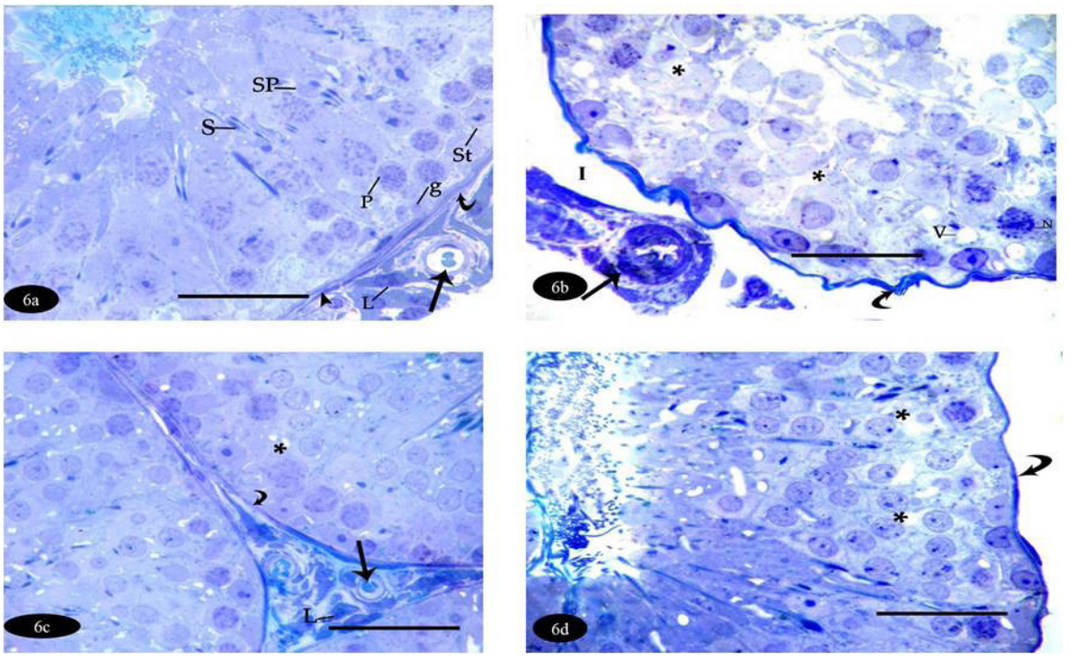

Fig. 6: a): A photomicrograph of a semithin section in the testis of a control 58 days albino rat revealing a seminiferous tubule lined by spermatogonia (g), primary spermatocyte (P), and spermatid (SP). The sperm heads (S). Sertoli cell (St) is seen lying on regular basement membrane (curved arrow) ensheathed by flat myoid cell (arrow head). Interstitium contains clusters of Leydig cells (L) and blood vessel (arrow) b): AgNPs treated subgroup there is separation between germinal epithelial cells (star) and many vacuoles (V), resting on irregular basement membrane (curved arrow). Some cells exhibit deeply stained nuclei (N) with wide interstitium (I) containing leydig cells and blood vessel (arrow) with a thick wall. c): AgNPs + vit E treated subgroup reveal regular basement membrane (curved arrow) with few spaces (star) between germinal epithelial cells. Apparently normal clusters of leydig cells (L) and blood vessels (arrow) are noticed. d): Follow up group shows slightly irregular basement membrane (curved arrow) with many spaces (star) in-between germinal epithelial cells (Toluidine blue x 1000 scale bar 30µm).

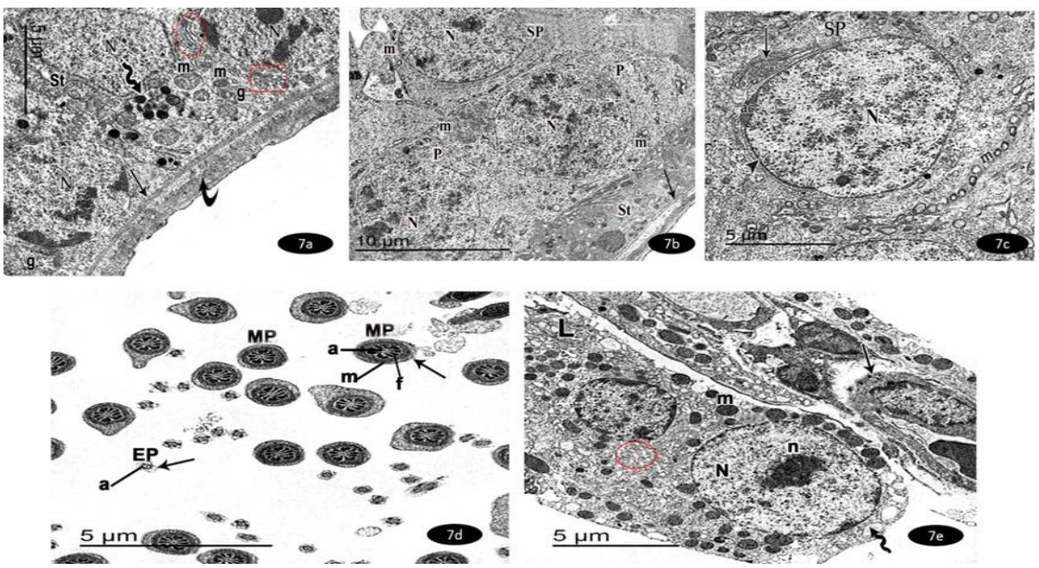

Fig. 7: a): An electron micrograph from 58 days control group showing spermatogonia (g) lying on regular basement membrane (arrow) ensheathed by myoid cell (curved arrow). Spermatogonia have rounded nuclei (N) its cytoplasm has cisternae of endoplasmic reticulum (circle) and golgi apparatus (rectangle), mitochondria (m). Sertoli cell (St) appears with euchromatic nucleus (N), mitochondria (m) and lysosomes (zigzag arrow) X6600. b): Sertoli cell (St) resting on regular basement membrane (arrow). Primary spermatocytes (P) appear with large rounded nuclei (N). Its cytoplasm contains mitochondria (m). Spermatid (SP) appears with large ovoid euchromatic nuclei (N). Cytoplasm contains numerous peripherally situated mitochondria (m) X6400. c): Late spermatid (SP) with ovoid euochromatic nucleus (N) and acrosomal cap (arrow head) with well-formed acrosomal vesicle (ac). Flattened Golgi saccules (arrow) and peripherally situated mitochondria (m) X10000. d): Cross sections in the middle pieces (MP) and end pieces (EP) of sperms. All pieces have central axoneme (a). In the middle piece, the axoneme is surrounded by nine thick course dense fibers (f), mitochondrial sheaths (m) and cell membrane (arrow). In the end piece, the axoneme is surrounded by cell membrane only (arrow) X12600. e): Leydig (L) contains spherical nucleus (N) and prominent nucleolus (n) its cytoplasm contains cisternae of smooth endoplasmic reticulum (circle), mitochondria (m) and many lipid droplets (zigzag arrow). A blood capillary (arrow) is also noticed X8400.

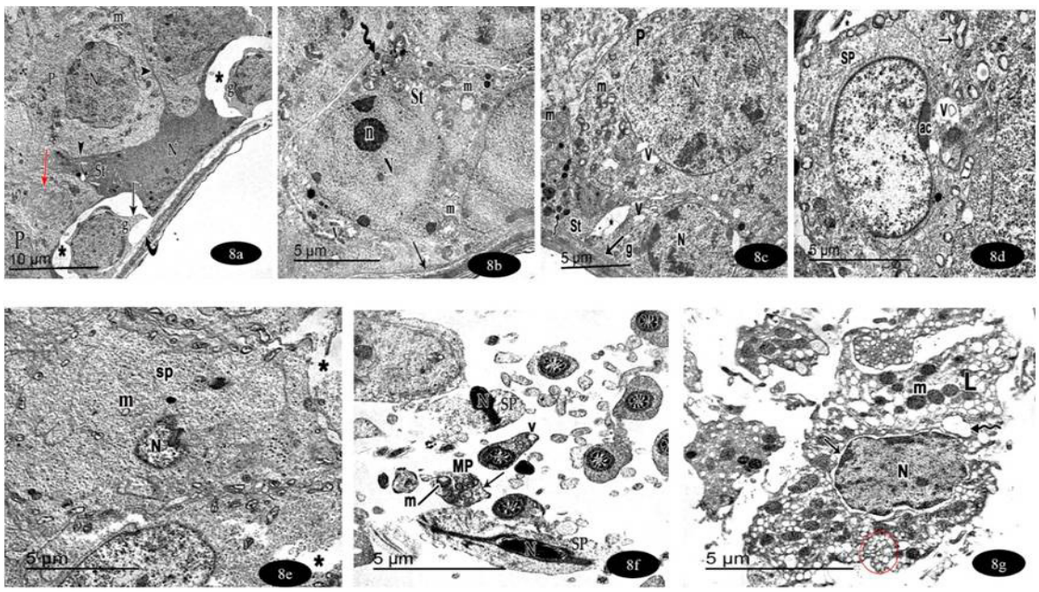

Fig. 8: a): An electron micrograph of AgNPs treated subgroup showing spermatogonia (g) with irregular cell membrane (arrow) partially separated from basement membrane (curved arrow) and from surrounding cells (star). A primary spermatocyte (P) has irregular shape nucleus (N) and disorganized mitochondria (m), disorganized golgi apparatus (red arrow). Sertoli cell (St) with electron dense nucleus (N) is seen sending cytoplasmic processes (arrow head) in-between germ cells with cytoplasmic vacuoles (V) X5000.
b): Sertoli cell (St) resting on basement membrane (arrow) with euchromatic nucleus (N) and prominent nucleolus (n). Its cytoplasm contains mitochondria (m) with disrupted cristae, lysosomes (zigzag arrow) and some vacuoles (V) X9600. c): Spermatogonium (g) and profile of sertoli cell (St) are resting on basement membrane (arrow). Spermatogonium has heterochromatic nucleus (N) and cytoplasmic vacuoles (V). A primary spermatocyte (P) also appears with large nucleus (N). Its cytoplasm contains mitochondria (m) and vacuoles (V). Sertoli cell has mitochondria (m) with disrupted cristae and lysosomes (zigzag arrow). Little spaces (star) are also noticed in-between cells X8200. d): A late spermatid (SP) with formed acrosomal vesicle (ac). Some mitochondria have abnormal shape (arrow). Many vacuoles (V) and intercellular spaces (star) are also noticed X12600. e): A spermatid (SP) with eccentric nucleus (N). Its cytoplasm contains abnormally located mitochondria (m). Widened intercellular spaces are also noticed (star) X10000. f): Cross sections of middle pieces (MP) of mature sperms with swollen mitochondrial sheath (m) and irregular cell membrane (arrow). Some pieces showed cytoplasmic vacuoles (V). Late spermatids (SP) with pyriform electron dense nuclei (N) are also seen X12600. g): Leydig cell (L) having an irregular nucleus (N) with peripheral heterochromatin and widened nuclear envelope (arrow). Its cytoplasm contains mitochondria (m), lipid droplets (zigzag arrow) and dilated cisternae of smooth endoplasmic reticulum (circle) X12600.

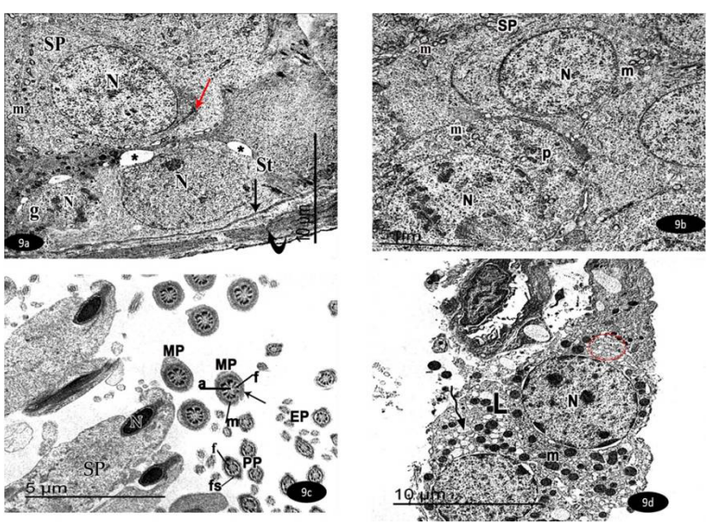

Fig. 9: a): An electron micrograph of AgNPs and vit. E treated subgroup showing spermatogonium (g) and sertoli cells (St) resting on slightly irregular basement membrane (arrow) ensheathed by myoid cells (curved arrow). Spermatogonium has heterochromatic nucleus (N). Sertoli cell has large euchromatic nucleus (N). A spermatid (SP) appears with rounded euchromatic nucleus (N) and peripherally situated mitochondria (m). Golgi apparatus (red arrow) also appears at one pole of nucleus. Little spaces (star) are also noticed in-between cells X4200. b): Primary spermatocyte (P) with large rounded nucleus (N), its cytoplasm contains mitochondria (m). Early spermatid (SP) appears with rounded euchromatic nucleus (N) and peripherally arranged mitochondria (m) X8000. c): Cross sections in sperms, in middle piece (MP), axoneme (a) is surrounded by nine thick course dense fibers (f), mitochondrial sheath (m) and cell membrane (arrow). In principal pieces (PP), axoneme is surrounded by dense fibers (f) and fibrous sheath (fs). End pieces of sperms (EP) and late spermatids (SP) with pyriform electron dense nuclei (N) are also seen X12600. d): Leydig cell (L) with oval regular nucleus (N), its Cytoplasm contains lipid droplets (zigzag arrow) with many cisternae of smooth endoplasmic reticulum (circle) and many mitochondria (m) X6700.

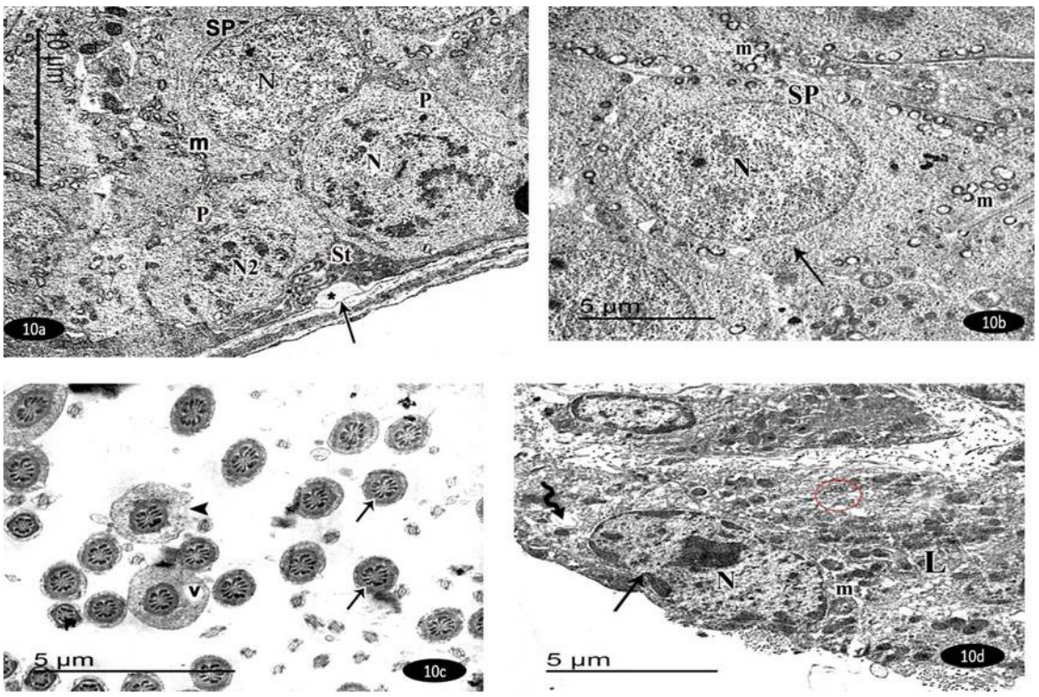

Fig. 10: a): An electron micrograph of follow up group showing primary spermatocytes (P) resting on basement membrane (arrow). One of them has euchromatic nucleus (N), other has shrunken nucleus (N2). Early spermatid (SP) with peripherally arranged mitochondria (m). Part of Sertoli cell (St) also appears with little separation (star) from basement membrane X4400. b): Spermatid (SP) with euchromatic nucleus (N). Its cytoplasm contains some peripherally situated mitochondria (m). Paranuclear mitochondria (arrow) also appear in the cytoplasm X8400. c): Cross sections of tails of sperms with some pieces having normal structure with well-organized axoneme and fibrous sheath (arrow). Some pieces show ruptured cell membrane (arrow head) and vacuoles (V) X12600. d): leydig cell (L) having oval nucleus (N) with irregular nuclear envelope (arrow). Its cytoplasm contains vacuolated mitochondria (m), dilated cisternae of SER (circle) and lipid droplets (zigzag arrow) X10200.

Table 1: Statistical analysis of serum testosterone level (ng/dl) by one-way ANOVA test.

Parameter	Control 23 days	Control 58 days	Group IIa	Group IIb	Group III	F	*P*
Serum testosterone (ng/dl)	0.33±0.08	3.4±0.67	3.16±0.55	3.45±0.44	3.73±0.81	61.25	<0.001**

Values are expressed as mean ± standard deviation (SD). **: Highly significant (*P*<0.001).

Table 2: Statistical analysis of height of germinal epithelium (µm) by one-way ANOVA test.

Parameter	Control 23 days	Control 58 days	Group IIa	Group IIb	Group III	F	*P*
Height of germinal epithelium (µm)	60±13.4	67.6±16.2	41.9±11.4	59.7±17.3	55.3±10.4	4.6	0.003*

Values are expressed as mean ± standard deviation (SD). **: Highly significant (*P*<0.001).

Table 3: Statistical analysis of Optical density of immune reaction to Bcl2 by one-way ANOVA test.

Parameter	Control 23 days	Control 58 days	Group IIa	Group IIb	Group III	F	*P*
Optical density of immunoreaction to Bcl2	5.41±1.4	6.31±1.82	1.51±0.45	4.9±1.07	4.08±1.14	21.09	<0.001**

Values are expressed as mean ± standard deviation (SD). **: Highly significant (*P*<0.001).

DISCUSSION

Silver nanoparticles with their antibacterial characteristics open new pathways to treat and prevent diseases. However, studies on AgNPs toxicity raise our doubts about their potential risk[20,21]. Children may be affected by daily products containing AgNPs because of their physiological functions, developmental stage and habits[22]. Gametogenesis is potentially affected by several environmental factors like exposure to toxicants infectious and inflammatory events which have a harmful impact on the germline and also negative consequences on fertility[23].

For this purpose, 56 healthy prepubertal male albino rats were utilized in this study. The choice of age 23 PND in our study relied on the fact that rodents are weaned at PND 21. After that, they begin to undergo sexual maturation[24]. Also age 58 days was chosen to scarify rats in accordance to studies that mentioned that the age of maturity varies between males, ranging from 40 days old to 76 days in rats[25].

In the present work, non-significant decrease in serum testosterone level in rats of AgNPs subgroup in comparison to the 58 day control group means that testosterone level was not altered by AgNP treatment with 50 µg /kg/d. The same result was found by other studies[12,10]. The latter clarified that the defects in spermatogenesis due to direct effects of AgNPs on spermatogenic cells led to the morphological insult and imperfect sperm function. It was also reported that the the concentration of AgNPs applied to the cells controls the viability of Leydig cell[26]. However, in other studies AgNPs appeared to affect Leydig cell function leading to increase in testosterone levels[27].

In AgNPs treated rats, shrunken and disorganized seminiferous tubules were explained by some authors as cytotoxicity of AgNPs is related to increased formation of reactive oxygen species (ROS), which could induce apoptosis[26]. Furthermore, the irregularities in the basal lamina could be secondary to myoid cells contraction or distorted seminiferous tubules[28].

Also, marked separation and spaces between germinal cells with apparent diminished layers of germinal epithelium. The latter, was confirmed statistically by highly significant decrease of the mean of epithelial height in AgNPs subgroup in comparison with the control group. Similar results were stated by another study used nanoparticles orally for 90 days and confirmed that degenerative alterations in seminiferous tubules showed that nanoparticles could directly inhibit spermatogenesis[29]. Few spermatozoa in tubules lumina were also seen in our study. This could be explained that by the fact that decrease in germinal stem cells number may probably have badly affected sperm production and lead to weakness in male fertility[30]. Other studies mentioned that the effect of nanoparticles on cell cycle or release of spermatozoa to the mid duct of seminiferous tubules led to a significant decrease in sperm stem cells[31].

Cytoplasmic vacuoles were detected within germ cells. These results were also confirmed by electron microscopic examination. Vacuolization may be effect of disturbances in membrane function, which results in influx of water and sodium. Also, Cellular swelling may be accompanied by cytoplasmic degeneration due to leakage of lysosomal hydrolytic enzymes[32]. Many cells reveal deeply stained nuclei. Similar results were stated by other studies using higher dose of AgNPs which explained that undue accumulation of ROS might be a triggering agent to apoptosis and autophagy of the germ cells[33,34].

Histological changes found in the interstitium were in agreement with other studies[33,35]. The latter declared that interstitial tissue damage may be associated with AgNPs deposition in the tissue. It was also observed that changes in the composition or volume of the interstitial fluid could also significantly affect the testicular function[36].

In the present work, results of immuno-histochemical stained sections for Bcl2 protein in AgNPs-treated subgroup; there were weak cytoplasmic immunoreactions in few cells of germinal epithelium and Leydig cells. These results were confirmed by statistical evaluation of optical density of Bcl2 was going with other authors who mentioned that ratio of Bax/Bcl-2 proteins plays a main role in mitochondrial membrane permeability, release of cytochrome C into cytoplasm and initiation of apoptosis[37,38].

Ultrastructurally, germ cells had irregular shape nuclei and vacuolated mitochondria. Oxidative injury to cell membranes results in great ionic imbalance, mitochondrial damage, and finally lysosomal activation[39]. Irregular membranes of early spermatids with abnormally arranged mitochondria. This is in accordance with[37].

Abnormalities in cross sections of middle piece of mature sperms were in harmony with another study on AgNPs[40]. Oxidative stress could lead to insufficient sperm production, motility disorders, increased abnormal forms and impaired function[41].

Interstitial Leydig cells had irregular cell membrane. Nuclei appeared with peripheral heterochromatin and widened nuclear envelope. Their cytoplasm had few lipid droplets. Similar results were reported by[42,26]. The latter admitted that cell viability was directly related to the concentration of AgNPs applied to the cells. Cell death was also noticed in a concentration-dependent manner. Moreover, higher levels of membrane leakage occur due to lactate dehydrogenase (LDH) release after cell membrane damage.

Irregular dilated cisternae of smooth endoplasmic reticulum (SER) found in our study was also described by other trials as vesicular SER, involving separated vesicles of variable diameters and dilated Golgi apparatus indicate typical adaptations of an activated steroidogenic cell. These alterations were not accompanied by changes in the level of total plasma testosterone when compared with the control group. This is also going with our study[43].

Vitamin E is a potent antioxidant believed to have a protective effect against oxidative stress which happens when the overproduction of ROS is not balanced by a satisfactory

level of antioxidants and so increase in free radicals which are toxic for biological membranes[44,45].

Serum testosterone level of the AgNPs and vit. E treated rats revealed non-significant decrease in the mean of serum testosterone level compared to control group. However, a prominent increase in the testosterone level of the testicular tissue of the rats in the vitamin E-administered group was observed by other authors[46]. Results of light microscopic stained sections of AgNPs and vit. E subgroup was confirmed by non-significant decrease of the mean of epithelial height in AgNPs and vit. E subgroup compared to the control group were found by other authors who found that vit. E improved testicular tissue but not as in the control group[46]. In another study on cerebellum, it was observed that vit. E could protect against ROS toxicity following AgNPs administration and counteract their toxic effects[47]. Vitamin E plays a vital role in reproductive health as it reduces testicular oxidative damage and its deficiency increased oxidative stress and decreased normal spermatogenesis and androgens production[48].

Results of immuno-histochemical stained sections for Bcl2 protein in the same group exhibited nearly normal cytoplasmic immunoreaction in the cells of germinal epithelium and Leydig cells. These were reported by other studies which detected that vit. E is effective in protection against apoptosis by decreasing caspase 3 expression and upregulating Bcl 2 expression, and by improving DNA oxidative destruction in bone marrow hemopoietic cells[49]. It was also reported that vit. E increases the Bcl2 level in mitochondria and decreases the level of Bcl2 like protein 4, suppresses release of cytochrome C, activates caspase 9 and caspase 3 and therefore, leads to inhibition of myocardial cell apoptosis[50].

Ultrastructural results were reported by other studies that the supplementation of vit. E assisted in the recovery of the structure of seminiferous tubules showing normal epithelium with a regular arrangement of germ cells within most phases of spermatogenesis process involving differentiation of spermatids[44,51].

Cross sections in the tails of sperms revealed the middle, principal and end pieces which had normal structure with well-organized axoneme. That could be explained as vit.E increased activity of sperm defense antioxidant system together with superoxide dismutase, glutathione peroxidase and catalase. This eventually led to increase sperm viability, count and motility[52].

Light and electron microscopic examination of follow up group showed incomplete recovery which was going with another study that found that AgNPs accumulated in a dose-dependent manner in the liver, spleen, testis, lung, kidneys, brain and blood. The authors also added that a four-week recovery period decreased the concentration of AgNPs in the liver, spleen, kidneys and testes, in comparison with the levels observed in the experimental group obtained at the end of the 4 weeks administration period of AgNPs[53]. In addition, the administrated AgNPs were cleared from liver, spleen and kidneys but slowly cleared or to some extent showing bio- persistency in organs, which have barriers like brain and testis[54,55].

CONCLUSION AND RECOMMENDATIONS

Prepubertal exposure to AgNPs had several deleterious effects on male reproductive organs even at small doses. They also had toxic cumulative effect stayed after 4 weeks of stoppage of adminsteration. Therefore, it is critical to understand their nature and origin of toxicity. However, these changes could be limited by vit. E supplementation. So, it is recommended to promote health awareness, limit exposure to such materials and encourage supplementation of vit. E. The scientific community should dedicate attention to establish protocols for toxicity elimination, in order to accurately determine the potential threats of AgNPs applications mainly on reproductive organs.

ABBREVIATIONS

Silver nanoparticles **(AgNPs)**; Vitamin E **(Vit.E)**; Nanoparticles **(NPs)**; Polyvinylpyrrolidone **(PVP)**; Post Natal Day **(PND)**.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Cattaneo AG, Gornati R, Sabbioni E, Chiriva-Internati M, Cobos E, Jenkins MR, Bernardini G: Nanotechnology and human health: risks and benefits. Journal of applied Toxicology. (2010) 30(8), 730-744.

2. Bartlomiejczyk T, Lankoff A, Kruszewski M, Szumiel I: Silver nanoparticles–allies or adversaries? Annals of Agricultural and Environmental Medicine. (2013) 20(1).

3. Emam HE, Saleh NH, Nagy KS, Zahran MK: Functionalization of medical cotton by direct incorporation of Silver nanoparticles. International journal of biological macromolecules. (2015)78: 249-256.

4. Ahamed M, AlSalhi MS, Siddiqui MKJ: Silver nanoparticle applications and human health. Clinica chimica acta. (2010) 411(23-24), 1841-1848.

5. Asare N, Instanes C, Sandberg WJ, Refsnes M, Schwarze P, Kruszewski M, Brunborg G: Cytotoxic and genotoxic effects of silver nanoparticles in testicular cells. Toxicology. (2012) 291(1-3), 65-72.

6. Rizvi S, Raza ST, Ahmed F, Ahmad A, Abbas S, Mahdi F: The Role of Vitamin E in Human Health and Some Diseases. Sultan Qaboos University Medical Journal. (2014) 14(2), 157–165.

7. Galli F, Azzi A, Birringer M, Cook-Mills JM, Eggersdorfer M, Frank J, Özer NK: Vitamin E: Emerging aspects and new directions. Free Radical Biology and Medicine. (2017) 102, 16-36.

8. Howard AC, Anna K, McNeil AK, McNeil PL: Promotion of plasma membrane repair by vitamin E. Nat Commun. (2011) 20, 597.
9. Ourique GM, Saccol EM, Pês TS, Glanzner WG, Schiefelbein SH, Woehl VM, Baldisserotto B, Gonçalves PB, Barreto KP: Protective effect of vitamin E on sperm motility and oxidative stress in valproic acid treated rats. Food and Chemical Toxicology. (2016) 95, 159-167.
10. Mathias FT, Romano RM, Kizys MM, Kasamatsu T, Giannocco G, Chiamolera MI, Romano MA: Daily exposure to silver nanoparticles during prepubertal development decreases adult sperm and reproductive parameters. Nanotoxicology. (2015) 9(1), 64-70.
11. Fang Y, Zhou Y, Zhong Y, Gao X, Tan T: Effect of vitamin E on reproductive functions and anti-oxidant activity of adolescent male miceexposed to bisphenol A. (Wei Sheng Yan Jiu.).(2013) 42(1), 18-22.
12. Sleiman HK, Romano RM, Oliveira CAD, Romano MA: Effects of prepubertal exposure to silver nanoparticles on reproductive parameters in adult male Wistar rats. Journal of Toxicology and Environmental Health, Part A. (2013) 76(17), 1023-1032.
13. Wen L, Gao Q, Ma CW, Ge Y, You L, Liu RH, Liu D: Effect of polysaccharides from Tremella fuciformis on UV-induced photoaging. Journal of Functional Foods. (2016) 20, 400-410.
14. Teilmann AC, Madsen AN, Holst B, Hau J, Rozell B, Abelson KSP: Physiological and pathological impact of blood sampling by retro-bulbar sinus puncture and facial vein phlebotomy in laboratory mice. PLoS ONE. (2014) 9(11): 1-19.
15. Bancroft J, Layton C: The Hematoxylin and eosin. Theory & Practice of histological techniques. 7th ed., Churchill Livingstone of El Sevier. Philadelphia. (2013) 173-214.
16. Ramos-Vara JA, Miller MA: When tissue antigens and antibodies get along: revisiting the technical aspects of immunohistochemistry—the red, brown, and blue technique. Veterinarypathology. (2014) 51(1), 42-87.
17. Ayache J, Beaunier L, Boumendil J, Ehret G, Laub D: Sample preparation handbook for transmission electron microscopy: techniques, Springer Science & Business Media. (2010) (Vol. 2).
18. Hayat ME: Basic techniques for transmission electron microscopy. Elsevier 2nd ed. (2012) pp.8-125.
19. Petrie A, Sabin C: Basic techniques for analysing data. In: Medical Statistics at a Glance. John Wiley & Sons. Ltd. 4th edn, (2019) pp. 51–54.
20. Tran QH, Le AT: Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives. Advances in Natural Sciences: Nanoscience and Nanotechnology. (2013) 4(3), 033001.
21. El Badawy AM, Silva RG, Morris B, Scheckel KG, Suidan MT, Tolaymat TM: Surface charge-dependent toxicity of silver nanoparticles. Environmental science & technology. (2010) 45(1), 283-287.
22. Tulve NS, Stefaniak AB, Vance ME, Rogers K, Mwilu S, LeBouf RF, Marr LC: Characterization of silver nanoparticles in selected consumer products and its relevance for predicting children's potential exposures. International journal of hygiene and environmental health. (2015) 218(3), 345-357.
23. Schagdarsurengin U, Western P, Steger K, Meinhardt A: Developmental origins of male subfertility: role of infection, inflammation, and environmental factors. In Seminars in immunopathology. Springer Berlin Heidelberg.765-781 ,6 ,38 (2016)
24. Sengupta PA: Scientific Review of Age Determination for a Laboratory Rat: How old is it in comparison with Human age? Biomed Int. (2012) 2, 81–90.
25. Lewis EM, Barnett Jr JF, Freshwater L, Hoberman AM, Christian MS: Sexual maturation data for Crl Sprague-Dawley rats: criteria and confounding factors. Drug and chemical toxicology. (2002) 25(4), 437-458.
26. Zhang XF, Gurunathan S, Kim JH: Effects of silver nanoparticles on neonatal testis development in mice. International journal of nanomedicine. (2015)10, 6243.
27. Garcia TX, Costa GM, Franca LR, Hofmann MC: Sub-acute intravenous administration of silver nanoparticles in male mice alters Leydig cell function and testosterone levels. Reproductive Toxicology. (2014) 45, 59-70.
28. Mohamed D, Saber A , Omar A, Soliman A: Effect of cadmium on the testes of adult albino rats and the ameliorating effect of zinc and vitamin E. British J. of Science. (2014) 11(1), 72-94.
29. Thakur M, Gupta H, Singh D, Mohanty IR, Maheswari U, Vanage, G, Joshi DS: Histopathological and ultrastructural effects of nanoparticles on rat testis following 90 days (Chronic study) of repeated oral administration. Journal of nanobiotechnology. (2014) 12(1), 42.
30. Ong C, Lee QY, Cai Y, Liu X, Ding J, Yung LYL, Baeg GH: Silver nanoparticles disrupt germline stem cell maintenance in the Drosophila testis. Scientific reports. (2016) 6, 20632.
31. Miresmaeili SM, Halvaei I, Fesahat F, Fallah A, Nikonahad N, Taherinejad M: Evaluating the role of silver nanoparticles on acrosomal reaction and spermatogenic cells in rat. Iranian journal of reproductive medicine. (2013) 11(5), 423.
32. Abdelhalim MAK: Gold nanoparticles administration induces disarray of heart muscle, hemorrhagic, chronic inflammatory cells infiltrated by small lymphocytes, cytoplasmic vacuolization and congested and dilated blood vessels. Lipids in health and disease. (2011) 10(1), 233.

33. Ahmed SM, Abdelrahman SA, Shalaby SM: Evaluating the effect of silver nanoparticles on testes of adult albino rats (histological, immunohistochemical and biochemical study). Journal of molecular histology. (2017) 48(1), 9-27.
34. Tan C, Lai S, Wu S, Hu S, Zhou L, Chen Y, Ji L: Nuclear permeable ruthenium (II) β-carboline complexes induce autophagy to antagonize mitochondrial-mediated apoptosis. Journal of medicinal chemistry. (2010) 53(21), 7613-7624.
35. Amin YM, Hawas AM, El-Batal AI, Elsayed SHHE: Evaluation of acute and subchronic toxicity of silver nanoparticles in normal and irradiated animals. British Journal of Pharmacology and Toxicology. (2015) 6(2), 22-38.
36. Lirdi LC, Stumpp T, Sasso-Cerri E, Miraglia SM: Amifostine protective effect on cisplatin-treated rat testis. The Anatomical Record. (2008) 291(7), 797-808.
37. Zielinska E, Zauszkiewicz-Pawlak A, Wojcik M, Inkielewicz-Stepniak I: Silver nanoparticles of different sizes induce a mixed type of programmed cell death in human pancreatic ductal adenocarcinoma. Oncotarget. (2018) 9(4), 4675.
38. Mullauer FB, Kessler JH, Medema JP: Betulinic acid induces cytochrome c release and apoptosis in a Bax/Bak-independent, permeability transition pore dependent fashion. Apoptosis.Springer. (2009) 14(2), 191-202.
39. Kumar V, Abbas AK, Aster JC: Robbins Basic Pathology. Elsevier; 9th edition, 2012.
40. Castellini C, Ruggeri S, Mattioli S, Bernardini G, Macchioni L, Moretti E, Collodel G: Long-term effects of silver nanoparticles on reproductive activity of rabbit buck. Systems biology in reproductive medicine. (2014) 60(3), 143-150.
41. Walczak–Jedrzejowska R, Wolski JK, Slowikowska–Hilczer J: The role of oxidative stress and antioxidants in male fertility. Central European journal of urology. (2013) 66(1), 60.
42. Han Z, Yan Q, Ge W, Liu ZG, Gurunathan S, De Felici M, Zhang XF: Cytotoxic effects of ZnO nanoparticles on mouse testicular cells. International journal of nanomedicine. (2016) 11, 5187.
43. Couto JA, Saraiva KL, Barros CD, Udrisar DP, Peixoto CA, Vieira, JSC, Wanderley MI: Effect of chronic treatment with Rosiglitazone on Leydig cell steroidogenesis in rats: In *vivo* and ex *vivo* studies. Reproductive Biology and Endocrinology. (2010) 8(1), 13.
44. Yue D, Yan L, Luo H, Xu X, Jin X: Effect of Vitamin E supplementation on semen quality and the testicular cell membranal and mitochondrial antioxidant abilities in Aohan fine-wool sheep. Anim Reprod Sci. (2010) 118, 217-222.
45. Domazetovic V, Marcucci G, Iantomasi T, Brandi ML, Vincenzini MT: Oxidative stress in bone remodeling: role of antioxidants. Clin Cases Miner Bone Metab. (2017) 14, 209–16.
46. Erdemli Z, Erdemli ME, Turkoz Y, Gul M, Yigitcan B, Gozukara Bag H: The effects of acrylamide and Vitamin E administration during pregnancy on adult rats testis. Andrologia. (2019) e13292.
47. Yin N, Yao X, Zhou Q, Faiola F, Jiang G: Vitamin E attenuates silver nanoparticle-induced effects on body weight and neurotoxicity in rats. Biochemical and Biophysical research communications. (2015) 458(2), 405-410.
48. Chandra AK, Chatterjee A, Ghosh R, Sarkar M: Vitamin E-supplementation protects chromium (VI)-induced spermatogenic and steroidogenic disorders in testicular tissues of rats. Food Chem Toxicol. (2010) 48, 972-979.
49. Jia YB, Jiang DM, Ren YZ, Liang ZH, Zhao ZQ, Wang YX: Inhibitory effects of vitamin E on osteocyte apoptosis and DNA oxidative damage in bone marrow hemopoietic cells at early stage of steroid-induced femoral head necrosis. Molecular medicine reports. (2017) 15(4), 1585-1592.
50. Qin F, Yan C, Patel R, Liu W, Dong E: Vitamins C and E attenuate apoptosis, beta adrenergic receptor desensitization, and sarcoplasmic reticular Ca2+ ATPase downregulation after myocardial infarction. Free Radic Biol Med. (2006) 40, 1827 1842.
51. Al-Zahrani S, Mohany M, Kandeal S, Badr G: Thymoquinone and vitamin E supplementation improve the reproductive characteristics of heat stressed male mice. JMPR. (2012) 6, 493-499.
52. Momeni HR, Eskandari N: Effect of vitamin E on sperm parameters and DNA integrity in sodium arsenite-treated rats. Iranian journal of reproductive medicine. (2012) 10(3), 249.
53. Lee JH, Sung JH, Ryu HR, Song KS, Song NW, Park HM, Yu IJ: Tissue distribution of gold and silver after subacute intravenous injection of co-administered gold and silver nanoparticles of similar sizes. Archives of toxicology. (2018) 92(4), 1393-1405.
54. Lee JH, Kim YS, Song KS, Ryu HR, Sung JH, Park JD, Park HM, Song NW, Shin BS, Marshak D, Ahn K, Lee HE, Yu IJ: Biopersistence of silver nanoparticles in tissues from Sprague–Dawley rats. Part Fibre Toxicol. (2013) 10, 36
55. Buzulukov YP, Gmoshinski IV, Antsiferova AA, Demin VA, Demin VF, Kashkarov PK: Accumulation of Silver Nanoparticles in Brain and Testes During Long-Term Ingestion to Mammals. In Nano Hybrids and Composites. Trans Tech Publications. (2017) 13,199-205.

الملخص العربى

تأثيرجزيئات الفضة الدقيقة علي خصية ذكور الجرذان المهق فى فترة ما قبل البلوغ والدور الوقائي المحتمل لفيتامين هـ (دراسة هستولوجية و هستوكيميائية مناعية)

خالد حامد المسلمي، محمد عبد الرحمن شاهين، نوره حسين عبد الحميد، فاطمة موسي الحسيني موسي

قسم الهستولوجيا- كلية الطب البشري - جامعة الزقازيق- الزقازيق - مصر

الخلفية: جزيئات الفضة الدقيقة واحدة من اكثر الجزيئات الدقيقة شهرة وشيوعا للاستخدام في منتجات المستهلك اليومية بالنسبة للكبار والاطفال علي حد سواء.

الهدف من الدراسه: تهدف هذه الدراسة الي دراسة تاثير التعرض لجزيئات الفضة الدقيقة قبل البلوغ علي التركيب النسيجي للخصيتين و دراسة الدور الوقائي المحتمل للإمداد بفيتامينه .

المواد والطرق: استخدمت جرذان المهق البيضاء في هذه التجربة والبالغة من العمر ثلاثة وعشرين يوما وقسمت الي ثلاث مجموعات: المجموعة الضابطة الاولي وقد تم تسقيمها الي مجموعتين فرعيتين: (المجموعة الفرعية الضابطة أ (السلبية) ولم تتلقي الجرذان أي علاج وتم ذبح البعض منها عند عمر ٢٣ يوما والاخري عند ٥٨ يوما. المجموعة الفرعية الضابطة ب (الايجابية) وقد تم تقسيمها الي مجموعتين فرعييتين. المجموعة الثانية وشملت المجموعة الفرعية لجزيئات الفضة الدقيقة وتم أعطائها بجرعة (٥٠ ميكروغرام / كجم / يوم) يوميًا لمدة ٣٥ يوما من عمر ٢٣ وحتي عمر ٥٨ يوما والمجموعة الفرعية المعالجة بجزيئات الفضة الدقيقة وفيتامين هـ : تلقت الجرذان جزيئات الفضة الدقيقة تزامنا مع فيتامين هـ بالانبوبة المعدية بجرعة (١٥٠ مغ / كغ / يوم) مذابة في زيت الذرة من عمر ٢٣ الي عمر ٥٨ يوما. واخيرا المجموعة الثالثة :مجموعة المتابعة فيها تم ترك بعض من الجرذان المعالجة بجزيئات الفضة الدقيقة بدون علاج الي عمر ٩٠ يوما.

النتائج: وجدت تغيرات هستولوجية كبيرة بنسيج الخصية في المجموعة المعالجة بجزيئات الفضة الدقيقة مقارنةً بالمجموعة الضابطة وفيتامين هـ ومجموعات المتابعة التي أظهرت تحسنًا كبيرًا. كما وجد انخفاض كبير بارتفاع الطبقة المبطنة للأنابيب المنوية في المجموعة الفرعية لجزيئات الفضة الدقيقة مقارنة بالمجموعه الضابطة وفيتامين هـ. كما اظهر الفحص الهستوكيميائي لعينات الخصية انخفاضا كبيرا للكثافة البصرية للتفاعل المناعي لبروتين ((Bcl٢ في المجموعة الفرعية لجزيئات الفضة الدقيقة مقارنة بالمجموعه الضابطة وفيتامين هـ.

الخلاصة: جزيئات الفضة الدقيقة لها آثار ضارة على الجهاز التناسلي الذكري والتي يمكن الحد منها عن طريق الامداد بفيتامين هـ والتقليل من التعرض لهذه المنتجات.