

## SPERM MALFORMED ULTRASTRUCTURE IN GOSSYPOL-TREATED RABBITS

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### SUMMARY

This study aimed to evaluate the effect of a sublethal dose of gossypol (20 mg/kg BW, every other day) on sperm malformed ultrastructure of male rabbits. The experiment lasted 8 weeks. Gossypol administration exhibited: severe deteriorations in the head, connecting piece and midpiece of the sperm; significant increases in the malformed acrosomes; dramatic destructions expressed as degeneration of cytoplasmic membrane and disappearance of some segmented columns; and significant increases in the incidence of cytoplasmic droplets. Other striking defects were also observed in the midpiece, especially disappearance of the cytoplasmic membrane, extensive vacuolation, loss of cristae and interruption in the continuity of the mitochondria along the length of the midpiece, as well as complete disappearance of some of outer dense fibers and of the nine-fiber doublets. In conclusion, gossypol treatment induced reproductive toxicity which alters the sperm morphogenesis and producing serious and deleterious malformed sperm ultrastructures.

**Keywords:** Gossypol, sperm ultrastructure, acrosome, axonemal fiber, mitochondrial sheath

### INTRODUCTION

Reduction in sperm motility has been reported in several animal species following treatment with gossypol (Randel *et al.*, 1992; Taha *et al.*, 2006). Chenoweth *et al.* (1994) suggested that impairment of sperm motility by gossypol treatment may be due to the structural damage to the sperm midpiece. Electron microscopy of bull sperm treated with gossypol revealed gaps in the mitochondrial helix of the midpiece (Velasquez-Pereira *et al.*, 1998), and the same was also reported in rats (Oka and Hrudka, 1982). Furthermore, electron microscopy of rat and bull spermatozoa revealed gossypol-induced extensive mitochondrial damage presented as extensive vacuolation and loss of cristae (Swan *et al.*, 1990; and Velasquez-Pereira *et al.*, 1998). Randel *et al.* (1992) suggested that this defect may be responsible for the observed reduction in sperm motility of animals treated with gossypol. Axonemal deficiencies are often the cause of lowered motility, progressive motility and fertility of the spermatozoa (Williamson *et al.*, 1984; Hancock and de Kretser, 1992), and abnormalities or reduction in the number of axonemal fibers could have significant effects on the progressive motility of the spermatozoon (Hancock and de Kretser, 1992; and Zavos *et al.*, 1998). One of the most striking changes in the axoneme of the gossypol-treated rats was the displacement of most of the outer dense fibers (Hoffer, 1983), as well as the disarrangement of the dense fibers and axial microtubules of the tail (Swan *et al.*, 1990).

Our recent studies clearly indicated that gossypol has pronounced hazardous effects on

seminal characteristics (Taha *et al.*, 2006) and seminal plasma constituents (Shaaban *et al.*, 2008; Taha *et al.*, 2008) in male rabbits, which may lead to deleterious productive and reproductive performance. A malformed acrosome of the gossypol-treated rat sperm was also noted indicating its dissolution and detachment from the underlining nucleus (Oka and Hrudka, 1982; and Hoffer, 1983). Moreover daily ingestion of gossypol provokes infertility in various animal species, including humans. The contraceptive effect of gossypol in man was discovered first in China, and still continues to be tested as a favorable candidate as a male contraceptive (Cui *et al.*, 2004). Thus the present study was carried out to evaluate the malformed ultrastructure arrangement of sperm in gossypol-treated rabbits.

### MATERIALS AND METHODS

#### *Animals and management:*

This study was carried out at the Department of Animal Production, Faculty of Agriculture, Alexandria University, Alexandria, Egypt. Experiments were carried out after the Departmental approval, and were done without any commercial profit purposes for the Authors or the Department. All procedures and experimental protocols were conducted in accordance with the "Guide for the Care and Use of Laboratory Animal" prepared by the Institute for Laboratory Animals Resources (ILAR) of the National Academy of Science, National Academy Press, Washington, D.C. (1996). The present experiment was carried out to study the effect

of gossypol on malformed ultrastructure arrangement of rabbit sperm. Rabbits were chosen as an experimental animal owing to the fact that they are excellent model for reproductive toxicological studies. This is because the male rabbit is the smallest, least expensive animal that can be ejaculated with an artificial vagina, where serial semen samples could be obtained for biochemical and fertility evaluations (Foote and Carney, 2000). Gossypol was extracted from cottonseeds and purified according to Boatner (1948) as described by Taha *et al.* (2006). Gossypol was firstly dissolved in acetone then in corn oil to give a concentration of 20 mg/ml; this mixture was freshly prepared on the day of dosing.

Ten mature male New Zealand White rabbits (from the Agricultural Experimental Station, Faculty of Agriculture, Alexandria University, Egypt) aged 6-8 months and weighing  $2.68 \pm 0.14$  kg at the beginning of the experiment were used during the reproduction season (starting in September to avoid summer heat stress, as rabbit semen characteristics vary among seasons where increasing ambient temperature adversely affects semen quality (Hafez and Hafez, 2000)). The rabbits were individually housed in cages. Feed and water were provided *ad libitum*. The animals were fed pellets consisting of (per kg) 330 g berseem (*Trifolium alexandrinum*) hay, 170 g soybean meal, 165 g grounded corn, 160 g barley, 120 g wheat bran, 38 g molasses, 10 g salt, 4 g dicalcium phosphate, and 3 g vitamins. The chemical analysis of the diet according to Association of Official Analytical Chemists (1995) indicated that it contained (per kg) 175 g crude protein, 140 g crude fiber and 27 g fat. All animals were allowed to adjust to their new environment and tested for semen quality through three weeks before the experiment began.

#### **Experimental design:**

The rabbits were randomly divided into two groups of five animals each, and were assigned at random to one of the following treatments: the first group served as control (the animals were given an equivalent dose of the vehicle consisting of corn oil + acetone); the second group was used to study the effect of gossypol (1/20 LD<sub>50</sub>, 20 mg/kg BW). The proper dose for each rabbit was given orally with the help of a syringe directly into the esopharyngeal region. All animals were dosaged every other day throughout the treatment period, which extended for eight weeks (i.e. 56 days which is almost equal to the duration of spermatogenesis in the rabbit (52 days) [Swierstra and Foote, 1965]). Semen collection was carried out weekly from all animals throughout the 8-week experimental

period. Ejaculates were obtained using an artificial vagina and a teaser doe and seminal characteristics were evaluated (Taha *et al.*, 2006).

#### **Electron microscopy:**

At the end of the treatment period, spermatozoa obtained from the control and gossypol-treated groups were used to study the effect of gossypol on the ultrastructural features of rabbit sperm.

#### **Transmission electron microscopy:**

All chemicals used in this study were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA). Freshly collected semen specimens were washed twice by centrifugation (800 xg for 10 min) and resuspended using phosphate-buffered saline medium. Transmission electron microscopy (TEM) specimens were fixed in glutaraldehyde (3%) buffered with 0.15 M cacodylate at pH 7.4. They were post-fixed in osmium tetroxide (1%), dehydrated in graded alcohol solutions, and embedded in Epon 812. Ultra thin sections were cut with glass knife on a Leica EMKMR2 ultramicrotome, England, stained with uranyl acetate and lead citrate, and examined under a JEOL 100 CX transmission electron microscope (Japan).

#### **Scanning electron microscopy:**

Semen samples were processed and prepared for Scanning Electron Microscope (SEM) examination by the methods of Hafez and Kanagawa (1973). The dried samples were mounted on brass stubs and coated with gold using ion sputtering device JFC-1100E JEOL, Japan. The specimens were examined at 25 kv with JEOL JSM-5300 Scanning Electron Microscope (Japan).

#### **Statistical analysis:**

Data were analyzed using generalized linear model procedure (SAS, 1999) by two-way ANOVA. Comparisons among main effects were tested using LSD<sub>05</sub> of SAS (1999).

## **RESULTS**

Data on the ultrastructural assessment of spermatozoa from control and gossypol-treated male rabbits are indicated in Table 1. The ultrastructural features of the spermatozoa obtained from these animals at the end of treatment period are presented in Figs. 1 to 5.

#### **Acrosomal defects:**

Significant increases ( $P < 0.01$ ) in the numbers of reacted acrosomes were noted following gossypol treatment (Table 1). Electron micrographs (Figures 1, A and C) display a normal rabbit sperm showing an intact acrosome; while the malformed

acrosome of gossypol-treated rabbit sperm (Figures 1, B and D) reveal its dissolution and detachment from the underlying nucleus.

#### **Connecting piece anomalous:**

Gossypol administration exhibited severe deteriorations in the connecting piece (Table 1), which was illustrated by transmission electron micrographs. Normal rabbit sperm revealing intact connecting piece with normal appearance of cytoplasmic membrane and mitochondrial sheath is displayed in Figure 2A, while gossypol-treated rabbit sperm exhibited lesion of the cytoplasmic membrane, disappearance of some segmented columns (Figure 2B) and degeneration in the mitochondrial sheath near the connecting piece (Figure 2C).

#### **Midpiece defects:**

The most striking changes observed in the sperm of gossypol-treated rabbits were defects in the midpiece (Table 1), especially degeneration of the mitochondrial sheath. Transmission electron micrographs of longitudinal sections of normal rabbit sperm through midpiece and principal piece (Figure 3A) indicated normal appearance of cytoplasmic membrane and mitochondrial sheath; while sperm of the gossypol-treated rabbits (Figures 3, B and C) revealed disappearance of cytoplasmic membrane and interruption in the continuity of the mitochondria along the length of the midpiece (Figure 3C) but the principal piece seems normal (Figure 3B). On the other hand, the ultrastructural arrangement of cross section in the midpiece of normal rabbit sperm (Figure 4A) displayed that cytoplasmic membrane, mitochondrial sheath, outer dense fibers and axoneme are all normal in their appearance. However, sperm of gossypol-treated rabbits (Figure 4, B and C) revealed rupture of cytoplasmic membrane and extensive vacuolation of mitochondria (Figure 4C). The most severe abnormality noted in the axoneme of sperm from gossypol-treated rabbits was the complete disappearance of some of the nine-fiber doublets and outer dense fibers (Figure 4C).

#### **Cytoplasmic droplets:**

Gossypol treatment was also associated with significantly higher ( $P < 0.01$ ) incidence of cytoplasmic droplets (Table 1). Scanning electron micrographs of the gossypol-treated rabbit sperm revealed the presence of cytoplasmic droplet (Figure 5A). Transmission electron micrographs of longitudinal (Figure 5C) and cross-section (Figure 5B) of the cytoplasmic droplet, surrounding the midpiece clearly defined the extensive vacuolation and loss of cristae along the length of the

mitochondrial sheath. The presence of numerous large vesicles in the cytoplasmic droplet and its hollow appearance was also observed (Figures 5, B and C).

## **DISCUSSION**

To express its fertility potential and fulfill its reproductive function, the spermatozoa must be viable and must possess an efficient motor apparatus to drive them through the relatively large distance separating the vagina from the oviduct, and an intact acrosome to acquire fertilizing competence through capacitation. At the ultrastructural level, the sperm tail provides the spermatozoon with means of motility and is composed of the neck and the middle, principal, and end-pieces. The entire length of the sperm tail or axoneme is composed of nine pairs (doublets) of filaments or microtubules that are arranged radially around two central filaments (9+2 arrangement). This arrangement of filaments is surrounded by nine outer dense fibers. The outer dense fibers appear to be associated with the nine pairs of filaments. Furthermore, the entire midpiece is covered by mitochondria that generate the energy needed for sperm motility.

The toxicity of gossypol on rabbit spermatozoa had not been previously evaluated at the ultrastructural level. In the present study, the effect of gossypol on sperm structures and specifically on the axoneme (of the sperm tail) at the level of the middle and principal-pieces was evaluated. The study revealed a malformed acrosome of the gossypol-treated rabbit sperm indicating its dissolution and detachment from the underlying nucleus (Figures 1, B and D), which is in agreement with previous studies in other species (Oka and Hrudka, 1982; Hoffer, 1983).

Several hypotheses had been proposed to explain the detrimental effects of gossypol at the ultrastructural level of spermatozoa: first, structural integrity of numerous sperm structures depends on S-S cross-linked polypeptides. Disturbance of this bond may be associated with damage to the midpiece mitochondria (Baccetti *et al.*, 1986); second, damage due to lipid peroxidation in human sperm has been associated with loss of membrane integrity (Aitken and Fisher, 1994) and; third, gossypol promotes the formation of reactive oxygen species (Barhouni and Burghardt, 1996).

Reduction in sperm motility has been reported in several animal species treated with gossypol (Randel *et al.*, 1992; and Wang *et al.*, 1992). Chenoweth *et al.* (1994) suggested that motility may be impaired by structural damage to the sperm midpiece in animals treated with

gossypol. Electron microscopy of bull sperm treated with gossypol revealed gaps in the mitochondrial helix of the midpiece (Velasquez-Pereira *et al.*, 1998), as well as in rats (Oka and Hrudka, 1982). Furthermore, electron microscopy revealed extensive mitochondrial damage which was presented as extensive vacuolation and loss of cristae (Velasquez-Pereira *et al.*, 1998). Also Randel *et al.* (1992) suggested that this defect may be responsible for the observed reduction in sperm motility in animals treated with gossypol. Collectively, these findings are in agreement with the present results which revealed an interruption in the continuity of the mitochondria along the length of the midpiece (Figures 3, B and C), besides several mitochondria were severely vacuolated or entirely demolished. The present observations are consistent with those of Hoffer (1983) and Swan *et al.* (1990).

Axonemal deficiencies are often the cause of lowered mass motility, progressive motility and fertility in spermatozoa (Williamson *et al.*, 1984; Hancock and de Kretser, 1992), where abnormalities or reduction in the number of axonemal fibers could have significant effects on the progressive motility of the spermatozoon (Hancock and de Kretser, 1992; Zavos *et al.*, 1998). One of the most striking changes in the axoneme of gossypol-treated rats was the displaced outer dense fibers (Hoffer, 1983), in addition to the disarrangement of the outer dense fibers and axial microtubules of the tail (Rovan *et al.*, 1984; and Swan *et al.*, 1990). These findings are consistent with the present observations in which the most severe abnormality noted in the axoneme of spermatozoa from gossypol-treated specimens was the complete disappearance of one or more of the outer dense fibers and one or more of the nine fiber doublets (Figure 4C).

Outer dense fibers are composed of several prominent proteins which have been assembled along the axoneme by means of "leucine zippers" (Shao *et al.*, 1999). Furthermore, it has been demonstrated that all leucine residues in upstream leucine zippers are required for interaction between outer dense fibers proteins (Shao *et al.*, 1997). In addition, maximal incorporation of leucine into the outer dense fibers polypeptides occurs during spermiogenesis (Vera *et al.*, 1987). Moreover, comparison of sperm tail outer dense fibers proteins of rat, rabbit and humans indicated structural similarity of sperm tail outer dense fibers among these different species (Kim *et al.*, 1999). Leucine zipper, one of these transcription factors, is rich in basic amino acids (Lysine and Arginine) and contains four or five leucine residue spaces at

intervals of seven amino acids (Cooper, 1997). Our previous studies on the same animals (Taha *et al.*, 2008) revealed that treatment with the same dose of gossypol reduced the levels of lysine, arginine and leucine up to 50%. The severe reductions in the levels of these amino acids which represent the main contributors of the transcription factor (leucine zipper) seem to hinder the synthesis or function of this transcription factor which in turn disturbs the regulation of gene expression. These findings are consistent with previous studies in which gossypol induced inactivation of transcription factors in rat spermatogenic cells (Teng, 1992; and Teng *et al.*, 1995). Due to the importance of the leucine zippers in sperm tail outer dense fiber protein interactions, the reducing effect of gossypol on lysine, arginine and leucine could explain and might be the reason for the observed disappearance of the outer dense fibers. Previous reports indicated that the contents of tubulin and its motor protein (dynein) in spermatogenic cells are simultaneously reduced after gossypol treatment (Teng, 1995; and 1997). Also, gossypol causes inhibition of various enzyme activities involved in tubulin gene expression and tubulin synthesis (Strom-Hansen *et al.*, 1989; and Gonzalez-Garza *et al.*, 1993). In addition, gossypol alters the structure of intracellular membrane which impairs the m-RNA transportation system and reduces the rate of tubulin synthesis (Reyes *et al.*, 1984; and de Peyster *et al.*, 1986). Furthermore, gossypol binds to tubulin at a specific site and consequently prevents the tubulin from the assembling into microtubules (Medrano and Andreu, 1986). The unassembled free tubulin binds to nascent tubulin polypeptides on ribosomes, blocking further elongation and causing degradation of the tubulin m-RNA (Cleveland, 1989; Amos and Amos, 1991). These observations have validated gossypol as a potential inhibitor for the synthesis of contractile apparatus in the spermatogenic cell.

The present ultrastructural studies on gossypol-treated rabbits also reveal missing segments of sperm plasma membrane, which is consistent with several previous reports (Rovan *et al.*, 1984; and Swan *et al.*, 1990). Gossypol can alter the properties of lipid membranes that can modify protein function and structure (Cuéllar and Ramirez, 1993). Barhoumi and Burghardt (1996) reported that gossypol promoted the formation of reactive oxygen species and the depletion of glutathione in rat hepatocytes. Bender *et al.* (1988) reported a reduction in the antioxidants in the testes of rats fed gossypol. Damage due to lipid peroxidation in human sperm has been associated with loss of membrane integrity (Aitken and Fisher, 1994).

In mammalian spermatozoa cytoplasmic droplets of various sizes are noted in the neck region as one of the abnormalities of ejaculated spermatozoa. In most mammalian species the incidence of cytoplasmic droplets is relatively small, and during the maturation processes of the spermatozoon in the epididymis, the droplet migrates caudally and ultimately disappears. In the spermatozoa of domestic animals, the presence of a cytoplasmic droplet in the neck region is considered as a sign of immaturity. In the cauda epididymidis, the major absorption of the proteins synthesized in the preceding parts of the epididymidis to the migrating sperm cells takes place, but it is probably also the site of the synthesis of enzymes which destroy unwanted or undesirable components of the spermatozoa. Unwanted components already collect in the cytoplasmic droplets from the testis (where formation of the droplet begins). In bulls, cauda epididymidis is the site of initial synthesis of seminal ribonuclease (Matoušek *et al.*, 1981) which, apparently by adsorption, inactivates undesirable RNA in the cytoplasmic droplets (Matoušek *et al.*, 1980 a). The synthesis of seminal ribonuclease depends on the blood plasma testosterone concentration where reduced testosterone synthesis leads to loss of seminal ribonuclease synthesis in the cauda epididymidis (Matoušek *et al.*, 1980 a; Matoušek *et al.*, 1981). Another substance isolated from bull seminal plasma is phospholipid-binding protein (PBP), which releases the cytoplasmic droplets from epididymal sperm cells (Matoušek and Kysilka, 1980). The synthesis of PBP is dependent on the blood plasma testosterone concentration (Matoušek *et al.*, 1980 b). Functionally, seminal plasma PBP is not species-specific, so it releases the cytoplasmic droplets from ram, boar and rabbit sperm cells. The synthesis of both seminal ribonuclease and seminal phospholipid-binding protein are dependent on the blood plasma testosterone concentration. Thus the suppression of the synthesis of both seminal proteins by gossypol treatment seems to be attributed to the gossypol-induced reduction in testosterone concentration noted in our previous studies on rabbits (Taha *et al.*, 2006). This reduction in the synthesis of both proteins seems to suppress the release of cytoplasmic droplets from epididymal sperm cells (Figures 5, A - C).

It has been shown that the cytoplasmic droplet contains lipids, carbohydrates and lysosomal and glycolytic enzymes, and is made up of components of the endoplasmic reticulum mixed with remnants of the disintegrating Golgi complex in the residual cytoplasm (Dott and Dingle, 1968). Gomez *et*

*al.* (1996) reported that the link between semen quality and the reactive oxygen species (ROS) generation lies in the presence of excess residual cytoplasm. Thus when spermatogenesis is impaired, the cytoplasmic extrusion mechanisms are defective and spermatozoa are released from the germinal epithelium carrying surplus residual cytoplasm. Under these circumstances, the spermatozoa released during spermiation are thought to be immature and functionally defective (Huszar *et al.*, 1997). Therefore, when the cytosolic space is increased through the retention of excess residual cytoplasm, the resultant greater activity of the glucose-6-phosphate dehydrogenase leads to the excessive generation of NADPH and hence to the over-production of ROS. The latter then initiates a lipid peroxidation cascade and participates in a loss of sperm function (Aitken *et al.*, 1997).

In conclusion, gossypol induced sperm malformed ultrastructure expressed as sperm membrane injuries, acrosome reaction, gaps in the mitochondrial helix of the midpiece, disappearance of some segmented columns and reduction in the number of axonemal fibers. This could be partially attributed to reduction in the level of some amino acids which seems to hinder the synthesis or function of some transcription factors (leucine zipper). This in turn disturbs the regulation of gene expression as reflected in axonemal deficiencies. Furthermore, gossypol directly promotes the formation of reactive oxygen species (ROS) and indirectly impairs spermatogenesis resulting in deficiency in cytoplasmic extrusion mechanisms which leads to overproduction of ROS. Collectively, the excessive generation of ROS initiates a lipid peroxidation cascade.

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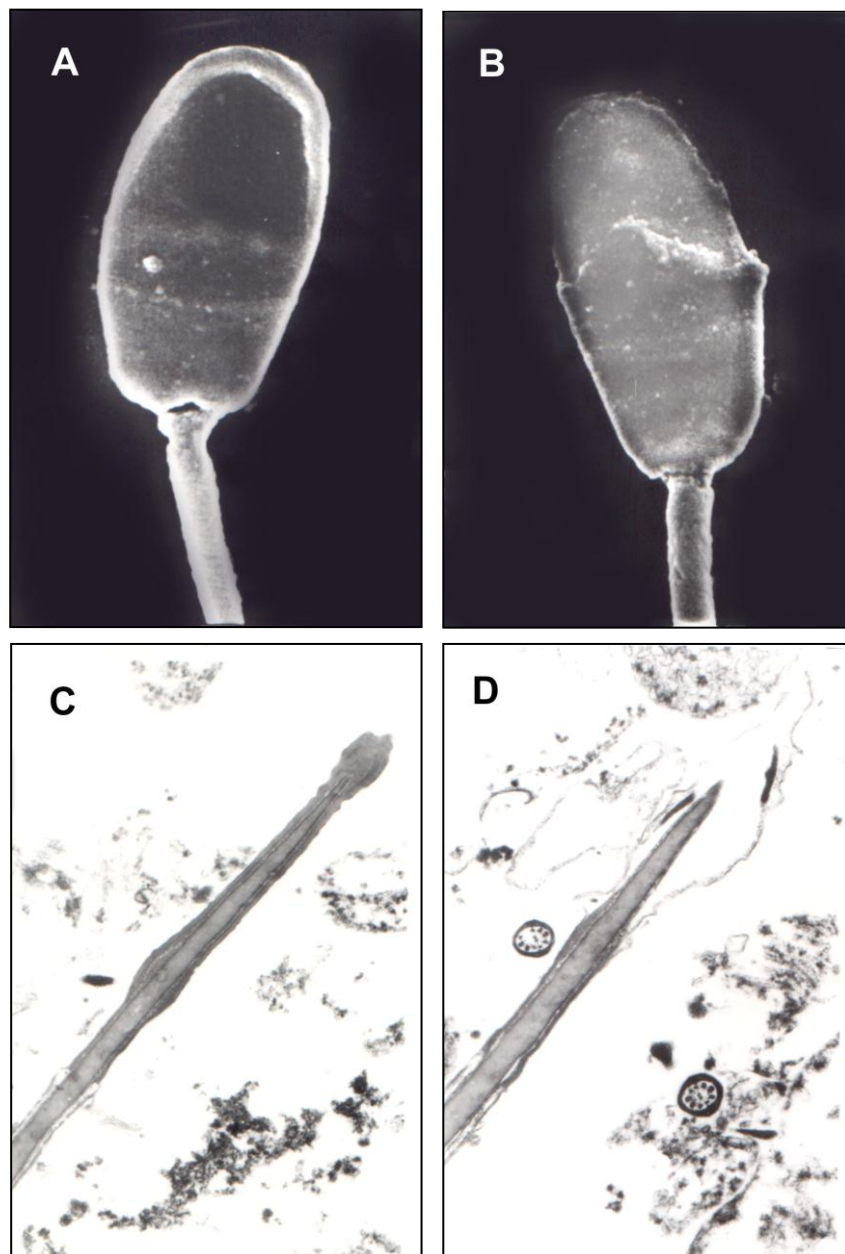
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**Table 1. Ultrastructural assessment of spermatozoa from control and gossypol-treated male rabbits**

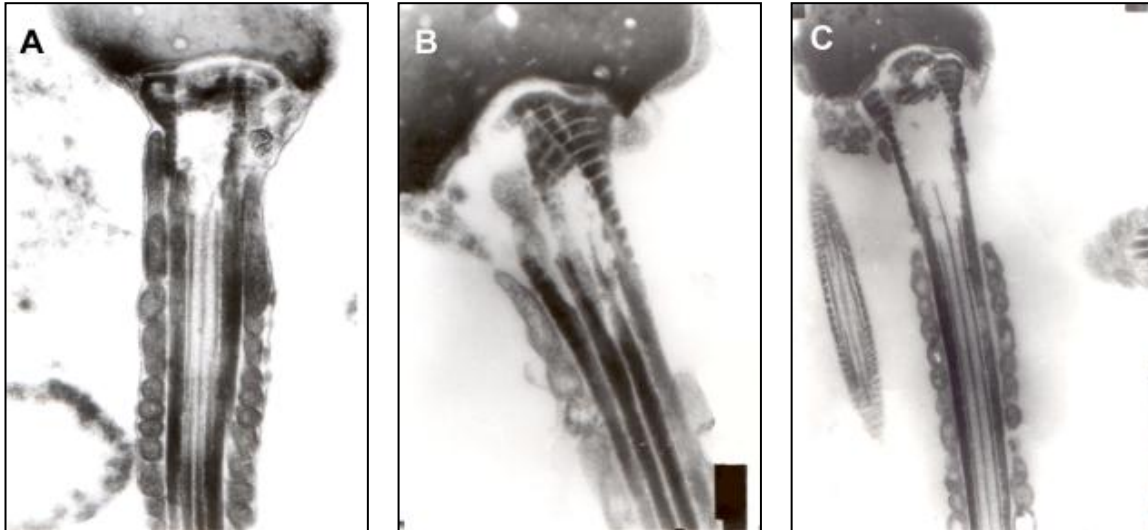
Semen parameters	Control	Gossypol-treated
Reacted acrosome (%)	11.68±0.44 <sup>b</sup>	25.79±1.63 <sup>a</sup>
Connecting piece	Intact	Lesioned
Segmented columns	Intact	Missing of some segmented columns
Cytoplasmic droplets (%)	24.90±1.49 <sup>b</sup>	50.74±2.31 <sup>a</sup>
Midpiece		
Cytoplasmic membrane	Intact	Ruptured or disappeared
Mitochondria	Intact	Vacuolated or disappeared
Axonemal fiber		
Coarse outer fiber	Intact	Missing of some coarse outer fiber
Fiber doublets	Intact	Missing of some fiber doublets
Central fibers	Intact	Intact
Principal piece	Normal	Normal

<sup>a, b</sup> Within rows, means with different superscript letters differ significantly ( $P < 0.01$ ).

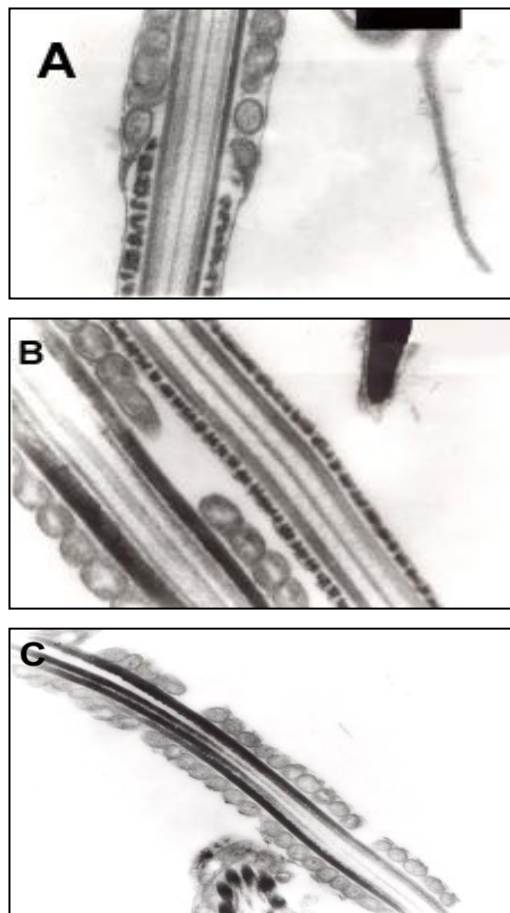


**Fig. 1. Scanning and transmission electron micrographs of control and gossypol-treated rabbit sperm. A, C) control rabbit sperm showing intact acrosome. B, D). Gossypol-treated rabbit sperm revealing reacted acrosome. Original magnification x10000 (A, B), x13000 (C) and x15000 (D).**

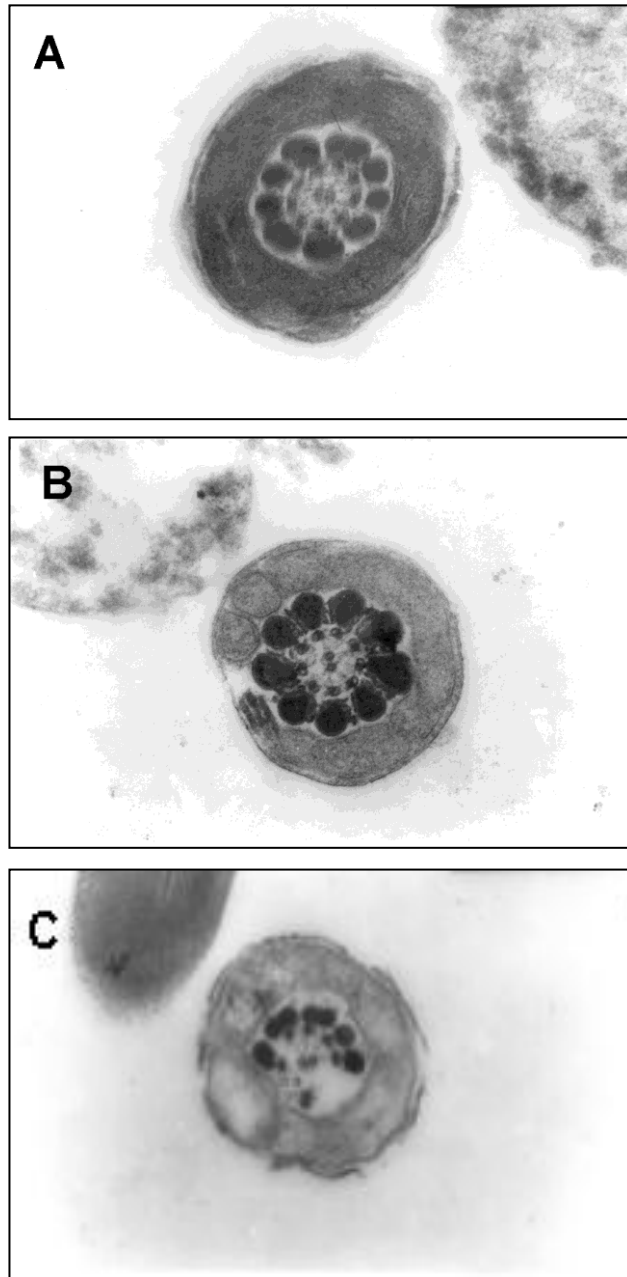




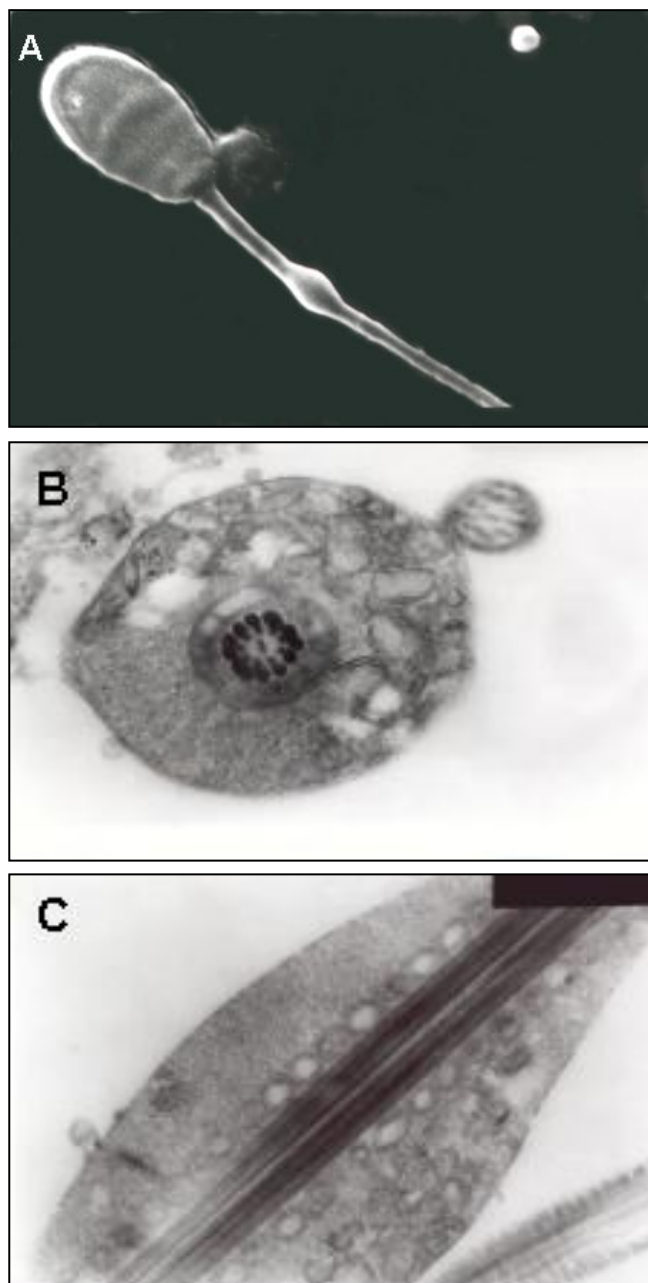
**Fig. 2.** Transmission electron micrographs of control and gossypol-treated rabbit sperm. A) Control rabbit sperm showing intact connecting piece and mitochondrial sheath. B, C) Gossypol-treated rabbit sperm illustrating lesion seen in the connecting piece expressed as degeneration of cytoplasmic membrane, and disappearance of some segmented columns (B) and complete removal of mitochondrial sheath near the neck region (C). Original magnification x25000 (A), x40000 (B) and x20000 (C).



**Fig. 3.** Ultrastructure of longitudinal section of rabbit sperm through midpiece and principal piece in control rabbit sperm (A) and in gossypol-treated sperm (B, C) illustrating gossypol-induced disappearance of cytoplasmic membrane and degeneration of mitochondria (C), but the principal piece seems normal (B) and degeneration of cytoplasmic membrane and interruption in the continuation of the mitochondria (C). Original magnification x40000 (A, B) and x20000 (C).



**Fig. 4.** Ultrastructural arrangement of cross-section in rabbit sperm midpiece in control rabbit sperm (A) revealing the normal appearance of mitochondrial sheath, outer dense fibers and axoneme; and in gossypol-treated rabbits (B, C) showing degeneration of mitochondria (B) and rupture of cytoplasmic membrane, extensive vacuolation of mitochondria and disappearance of some outer dense fibers and fiber doublets (C). Original magnification x50000 (A, B, C).



**Fig. 5.** Cytoplasmic droplets of gossypol-treated rabbit sperm. A) Overview of gossypol-treated rabbit sperm illustrating a cytoplasmic droplet. B, C) Transmission electron micrographs of cross (B) and longitudinal sections (C) of cytoplasmic droplet revealing extensive vacuolation and loss of cristae of mitochondria. Note the presence of numerous vesicles in the cytoplasmic droplet and its hollow appearance. Original magnification x5000 (A) and x30000 (B, C).

## التشوهات في التراكيب الدقيقة في الحيوانات المنوية في الأرناب المعاملة بالجوسيبول

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هدفت هذه الدراسة إلى تقييم تأثير جرعة تحت المميثة من الجوسيبول (٢٠ ملجرام / كجم من الوزن الحي يوم بعد يوم) على التشوهات في التراكيب الدقيقة في الحيوانات المنوية لذكور الأرناب. واستغرقت هذه الدراسة ثمانية أسابيع. المعاملة بالجوسيبول أظهرت أضراراً شديدة في منطقة الرأس والعنق والمنطقة الوسطى من الحيوان المنوي، و زيادة معنوية في تشوهات الأكروسوم، وتدمير شديد ظهر كتحلل للغشاء السيتوبلازمي، واختفاء بعض الأعمدة المفصصة في منطقة العنق، وزيادة معنوية في حدوث القطرة السيتوبلازمية. أيضاً لوحظت بعض الأضرار الشديدة الأخرى في القطعة المتوسطة خاصة اختفاء الغشاء السيتوبلازمي وحدوث تجايف شديدة وفقدان الحواجز في الميتوكوندريا وعدم استمرار وجود الميتوكوندريا على امتداد القطعة المتوسطة وأيضاً اختفاء كامل لبعض الألياف الكثيفة الخارجية وبعض أزواج الليبيفات التسعة. والخلاصة فإن المعاملة بالجوسيبول أحدثت سمية تناسلية غيرت الشكل التخليقي للحيوان المنوي وأحدثت أضراراً وتشوهات جسيمة في التراكيب الدقيقة للحيوانات المنوية.