Ultrastructure Study of Frozen-Thawed Spermatozoa of Friesian Bulls Treated by Certain Anthelmintic Drugs

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Abstract: Effect of three anthelmintic drugs (ivermectin, levamisole and albendazole) on the ultrastructure of spermatozoa of frozen- thawed semen Friesian bullswere used in the present study. 16 bulls were divided into four equal groups: group1, served as control untreated bulls (unfrozen and frozen semen); groups 2, 3 & 4 served as treated bulls with ivermectin (200µg/kg bw); levamisole (7.5mg/kg bw) and albendazole (10mg/kg bw), respectively. The drugs used at two therapeutic doses with 8weeks interval between first and second dose. Semen samples were collected at 8th and 16th weeks and evaluated to be frozen, and then after thawing of semen samples, they were processed to separate spermatozoa for TEM examination. Control spermatozoa (unfrozen) showed common normal architecture; head (with a flat nucleus and acrosomal cap), neck, mid and tail (principal and terminal) pieces surrounded by cell membrane, normal mitochondria, longitudinal fibers and axonemes. Frozen control semen elucidated few spermatozoa withslight detached or swollen plasma membranes surrounding normal nuclei. The treatment of bulls with either first or second dose of ivermectin showed slight swollen plasma membrane of spermatozoa, loosing acrosomes, distorted mitochondria and slight deformation of the axonemes. More deformation of spermatozoa was seen in the bulls treated with two doses of livamisole, while bulls treated with albendazole demonstrated severe damage of spermatozoa. Therefore, it could be recommended to treat bulls with ivermectin, while levamisole should be used careful. Albendazole treatment should be avoided.
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Key Wards: Anthelmintics – Bulls -Frozen spermatozoa - Ultrastructure

1. Introduction

There are many drugs used against internal and external parasites routinely administered in farm animals. Many studies focused on the effects of anthelmintic drugs that cause a transitory depression of fertility, suggest avoiding the treatment of animals during the mating season **(Raisinghani, 1992)**. Ivermectin, albendazole, and levamisole are the most used antiparasitic drugs in domestic animals (sheep, goats, cattle, horses and pigs) and used against a variety of nematodes as well as trematodes **(Corderodel, 1999; Al-Qarawi *et al.*, 2004).** They have considerable effects on the spermatozoa of male reproductive system, reduce semen quality (**Bakst *et al.*, 2006*;* Tag El-Dein *et al.*, 2011)** and reduce testosterone level **(Naoman, 2012).**

Ivermcetin is abroad spectrum anti-parasitic agent belonging to the avermactine family, It is highly acceptable anti-nematode and ecto-parasite drug within the breeding because of its broad range of activity and widely rage of safety. The veterinarian are used ivermcetin as a routine treatment against nematode. Levamisole is highly acceptable anti-nematodal drug because of its broad range of activity in a large number of hosts. This nematicide targets the nicotinergic acetylcholine receptor, resulting in depolarisation of neuronal and muscle cells **(Fleming *et al., 1997;* Culeuo *et al.*, 2004)**, skeletal and vascular abnormalities **(Teruel *et al.*, 2009)** Also, Benzimidazoles constitute one of the main groups of antihelmintics used clinically and they are the largest chemical family used to treat endoparasitic diseases in domestic animals. They inhibit the energetic metabolic of parasites and bind with tubulin, inhibiting its polymerisation and thus interfering with microtubule-dependent glucose uptake **(Mottier *et al.*, 2003)**.

Folador (2000) suggested that the treatment with avermectins didn't provoke any alteration in the reproductive parameters and ivermectin is able to induce increase in the body weight without changing the rate of food intake. In contrary, many authors recorded the undesirable effects of ivermectin on male fertility, as reduced semen concentration and sperm motility **(Tanyildizi and Bozkurt, 2002).** Ivermectin administration once weekly for 8 weeks induced a decrease in weights of the testis, epididymis and accessory sex organs and did not significantly change sperm characteristics. But, giving verapamil plus ivermectin significantly induced hazardous effects on

sperm - cell concentration, motility and sperm abnormalities **(El-Nahas and El-Ashmawy, 2008).**

Tag El-Dein *et al.* (2011) reported the treatment of the bulls with ivermectin at a level of 200µg/kg bw appeared more better post-thawing sperm motility, freezability of spermatozoa, as well as, the highest percentage of the intact acrosome, maintained DNA integrity and subsequent fertilizing efficiency of spermatozoa than the untreated (control) bulls or bulls treated with levamisole at a level of 7.5mg/kg or albendazole at a level of 10mg/kg either in the first or second dose with 8weeks interval.

Tabl *et al.* (2013) recorded the treatment of bulls with ivermectin improved the semen quality, total sperm output ejaculate and the activity of acid and alkaline phosphatases of seminal plasma, while albendazole recorded opposite results**.**

The considerable availability of raw material (spermatozoa) interest renders the present work attractive from an economic point view. Lack of studies on the effects of anthelmintic on cytological changes under farm conditions has a drawback to clearly monitor the productivity of such animals. For this reason, the purpose of this study is to shed some light on the direct effect, and to evaluate possible male reproductive health-related problems associated with the use of anthelmintic drugs. So the present investigation aimed to study the effect of three different anthelmintic drugs (ivermectin, levamisole and albendazole) on ultrastructure of frozen - thawed spermatozoa of Friesian bulls.

2.Materials and Methods

The present work was carried out at El-Karada Animal Production Research Station, El-Karada Village, Kafr El-Sheikh Governorate, located in the North Eastern part of the Nile Delta (31˚N), belonging to Animal Production Research Institute, Agricultural Research Center, Egypt during the period from May, 2009 to April, 2010.

1. Animals: Sixteen Friesian bulls with an initial live body weight of 380±10.2kg and aged 20 months were used in the present study.

2. Drugs: Ivermectin (paramectin®) and levamisoleHCl (levapan® 10%) were supplied by Pharma Swede- Egypt. Albendazole (deltazole 10%) was supplied by Delta Pharma, Egypt.

3. Experimental protocol: Bulls were randomly divided into four groups (4/each). Bulls in group 1 were untreated acting as control, while those in groups 2, 3 and 4 were treated with therapeutic dose of ivermectin (200µg/kg/bw, subcutaneously), levamisoleHCl (7.5mg/kg/bw, subcutaneously) and albendazole (10mg/kg/bw, orally), respectively. Dose repetition (the second dose) was administered after 8 weeks from the first one. The trail was continued for another 8 weeks, as a first dose according to **Benz *et al*. (1989)**.

4. Experimental procedures:

a. Semen collection*:* Semen samples were collected from all bull groups by means of an artificial vagina between 08.00 and 09.00 a.m. Two successive ejaculates were obtained from each bull at each day of collection (collection period of 8 weeks).

b. Freezing semen procedures:

b1. Semen extension: Semen was evaluated immediately after collection, then extended with Tris-yolk fructose (TYF) extender containing 3.028g trisaminomethane, 1.675 g citric acid anhydrous, 1.25 g fructose,7% glycerol, 20 ml egg yolk, 500 IU penicillin and 500 µg streptomycin added to 100 ml distilled water. The final extension rate pre-freezing was one semen: 20 extender **(Salisbury *et al.*, 1978).**

b2. Equilibration period: Extended semen was kept at 5°C for 4 hrs as an equilibration period. Then, the cooled semen was packaged in straws and frozen in liquid nitrogen (-196˚C) as the method described by **Salisbury *et al.* (1978)**.

b3. Evaluation of frozen-thawed semen: Frozen semen in straws was thawed by holding the straws at the closed end (not plugged end) and was dipped in water bath at 37° Cfor 30 seconds.

5. TEM preparation: After thawing of semen samples, 0.5 ml was taken from each sample and mixed with the fixed solution then centrifuged (3000 r.p.m), and the supernatants were removed. The remaining pellets containing spermatozoa were then rapidly fixed in 2.5% 0.1 M phosphate-buffered glutaraldehyde for 2hrs at 4°C **(Mcdowell and Trump, 1976)**. Spermatozoa were then washed in 0.1M phosphate buffer, fixed for 1 hr in 0.1M phosphate buffered 1% osmium tetroxide (at room temperature) and washed several times in the buffer. Dehydration was then performed in ascending series of ethyl alcohol. The dehydrated samples were treated with two changes of propylene oxide and transported to a mixture of propylene oxide and resin. The samples were embedded in epoxy resin mixture and left overnight. Two changes of resin were then applied and the samples were left in the oven at 60°C for 24 hrs for polymerization into plastic blocks. The blocks were sectioned at a thickness of 60-70 nm and mounted on copper grids. The grids were stained with uranyl acetate and lead citrate and left to dry **(Reynolds, 1963).** The grids were examined and photographed by using JEOL transmission electron microscope (TEM) at 80 KV in Faculty of Medicine, Tanta University, Egypt.

3. Results

1. Untreated control bulls (Group 1):

1.1. Unfrozen semen (Fresh semen):

The bulls in the control group showed common characteristic features of spermatozoa structure by using TEM: head, neck, mid piece and tail (principal and terminal pieces). The head is elongated with a flat nucleus; nuclear chromatin is moderately electron dense and homogenously distributed surrounded by intact plasma membrane (Figure 1). The mid piece contains spiral mitochondrial sheath surrounding the outer longitudinal fibrous sheath (nine bundles of microfilaments) and the axoneme (microtubules 9+2). The principal tail piece contains longitudinal fibrous sheath and inner cilia like structure typical microtubules arrangement (9+2). The end piece (treminal piece) has no fibrous sheath and is formed mainly by the axoneme surrounded by plasma membrane (Figure 1).

1. 2. Frozen semen:

The frozen thawed semen of untreated bulls at 8th week showed the spermatozoa with slight detached or swollen plasma membranes surrounding normal nuclei (Figure 2), normal appearance of mitochondria, normal longitudinal fibers and axonemes in mid and principal tail pieces (Figure 3). At 16th week of thawed semen, few spermatozoa elucidated obviously swollen plasma membranes (Figure 4), while the mid and tail pieces (principal and terminal pieces) were observed with normal intact architectures (Figure 5).

2. Ivermectin treated - bulls (Group 2):

The bulls treated with the first dose of ivermectin showed many spermatozoa of frozen- thawed semen with disintegration of the plasma membrane in few spermatozoa, others revealed with few and slight swollen areas of plasma membranes surrounding the sperms' head. Besides, numerous intact spermatozoa were seen with normal nuclei and intact acrosomal regions, normal mitochondria and longitudinal fibers in the mid pieces and approximately normal tail regions revealed also with common characteristics shape (Figs.6&7).The spermatozoa of the bulls treated with the second dose of ivermectin showed swollen and separation of plasma membranes surrounding normal nuclei, degeneration and loose of acrosomes, distorted mitochondria in mid piece and slight deformation of the axonemes in tail pieces (Figs. 8 & 9).

3. Levamisole treated - bulls (Group 3):

The spermatozoa of frozen- thawed bull semen treated with the first dose of levamisole showed increment of irregular, swollen and disintegration of sperms' head plasma membranes while the nuclei appeared with normal homogenous condensed chromatin (Figure 10). Lysis and rupture of the plasma membrane surrounding mid pieces of sperms, distortion of the mitochondria and longitudinal fibers were also observed. Slight deformation of axonemes was seen (Figures 10 & 11). After the treatment with a second dose of levamisole, the spermatozoa showed the same previous changes that appeared with the first dose except the distorted mitochondria and irregularly fused longitudinal fibers were obviously increased in mid pieces and slightly deformed axonemes in the tail pieces (Figures 12 & 13).

4. Albendazole treated - bulls (Group 4):

The spermatozoa of frozen- thawed semen of bulls treated with the first dose of albendazole showed with severe forms of membrane discontinuity, lysis, swollen and irregularly extended of plasma membrane around sperms' head and unhomogenous condensed chromatin (Figure 14). Axoneme (microtubules) and longitudinal fibers (microfilaments) were also degenerated and lost their arrangements (Figure 15).The bulls treated with the second dose of albendazole showed disturbances and damages in different pieces of spermatozoa more than that seen in the first dose. In addition, the increment of distorted mitochondria and axonemes was revealed (Figures 16 & 17).

In conclusion, the bulls treated with the first and second doses of albendazole demonstrated severe damage of spermatozoa comparing to ivermectin or levamisole treatment.

4. Discussion

Many studies focused on the effects of anthelmintic drugs that cause a transitory depression of fertility. Ivermectin, albendazole, and levamisole are the most used antiparasitic drugs in demostic animals **(Al-Qarawi *et al.*, 2004)).** They environmentally cause toxic damage to the testes, impact spermatogenesis reduced sperm production defective spermatozoa motility and impaired androgen production **(Anway *et al.*, 2005)**.

The present investigation showed slight swollen plasma membrane of few spermatozoa ofthe bulls treated with first dose of ivermectin resemble to the frozen control ones, while second dose demonstrated disturbances and disintegration of plasma membrane, loose acrosome, distorted mitochondria and slightly deformation of axoneme. The first and second doses of levamisole treatment showed the spermatozoa with swollen and disintegration of the plasma membrane surrounding head and mid piece, distorted mitochondrial structure and fused longitudinal fibers. However, severe damage of spermatozoa was seen at the first dose of albendazole and they demonstrated with lysis, swollen and discontinuity of cell membrane, unhomogenous condensed chromatin of the nuclei, distortion of mitochondria in mid piece, disintegration of longitudinal fibers (microfilaments) and degeneration of axonemes (microtubules).

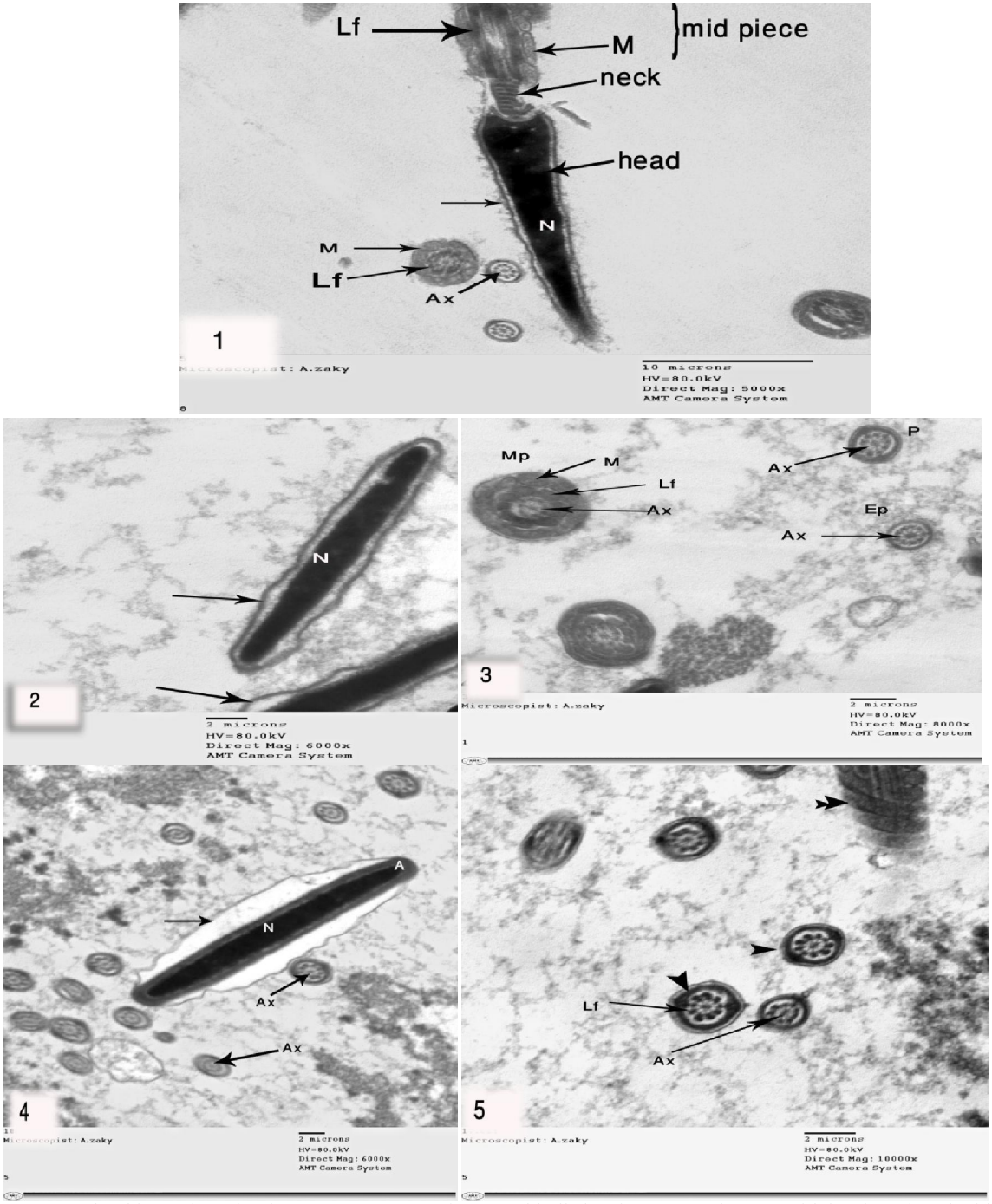

Fig. (1): Ultrathin section of unfrozen spermatozoa of a control bull showing a normal sperm with head, neck and mid piece. The head piece shows normal nucleus (N) surrounded by intact plasma membrane (arrow), and normal mid pieces are seen with mitochondria (M), longitudinal fibers (Lf) and axoneme (Ax) in tail piece, (x 5000). **Fig. (2):** Ultrathin section of spermatozoa of frozen- thawed semen of a control bull after 8 weeks of the beginning experiment showing head pieces with slightly detached or swollen plasma membranes (arrows) surrounding normal nuclei (N), (x 6000). **Fig. (3):** Ultrathin section of spermatozoa of frozen- thawed semen of a control bull after 8 weeks of the beginning experiment showing normal mid (Mp) with normal mitochondria (M) and longitudinal fibers (Lf),tail "principal (P) and end (Ep)" pieces with intact axonemes (Ax), (x 8000)**. Fig. (4):** Ultrathin section of spermatozoa of frozen- thawed semen of a control bull at 16th week showing an obvious swelling plasma membrane surrounding normal nucleus (N) in a spermatozoon head (arrow) and acrosome (A). Numerous normal axonemes (Ax) at terminal pieces are also seen, (x 6000). **Fig. (5):** Ultrathin section of spermatozoa of frozen- thawed semen of a control bull at 16th week showing normal intact principal pieces with intact plasma membranes (arrowheads), longitudinal fibers (Lf) and axoneme (Ax). A part of mid piece with normal mitochondria (double arrowhead) is also observed, (x 10000)

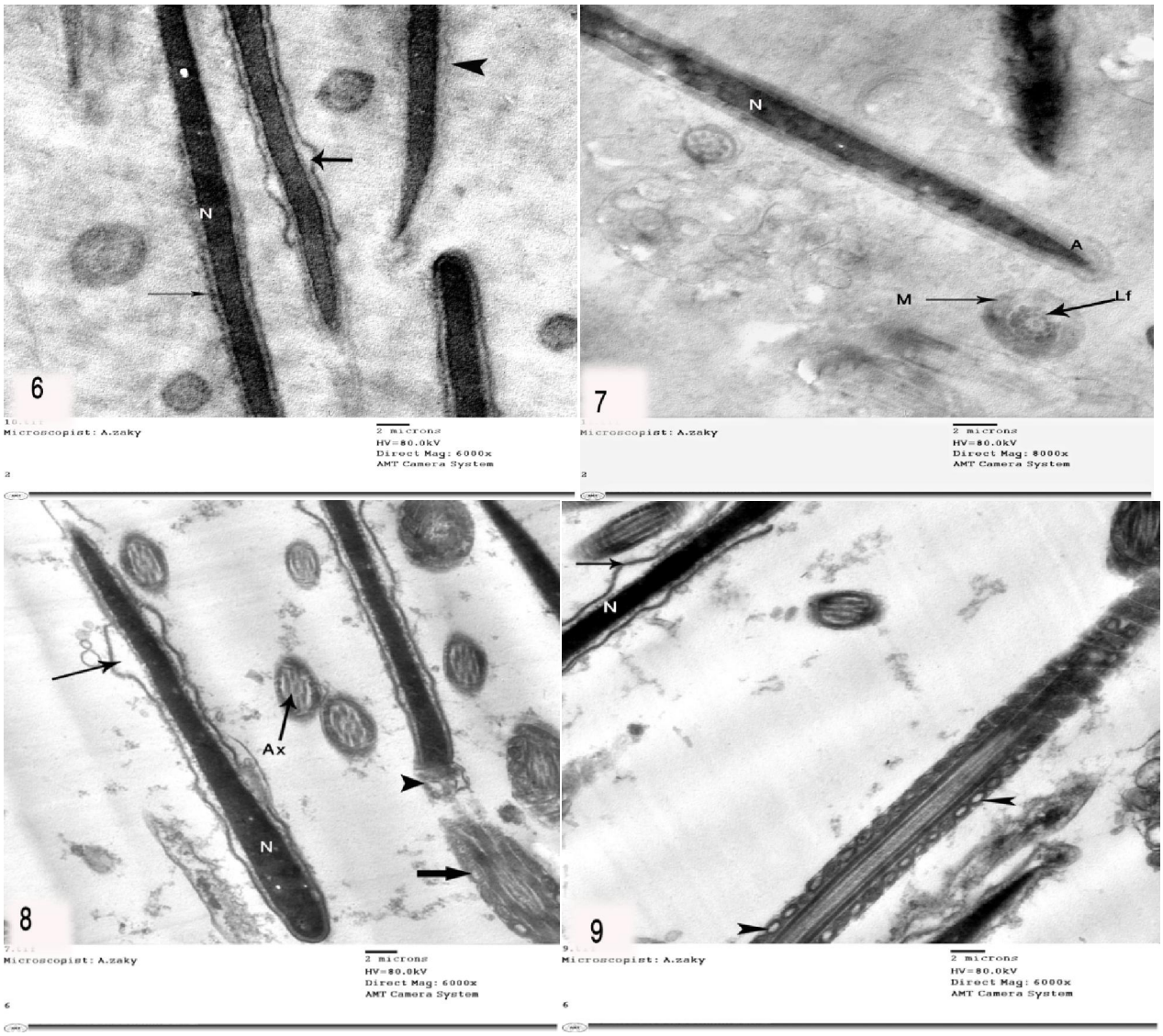

Fig. (6): Ultrathin section of spermatozoa of frozen-thawed semen of a bull treated with the first dose of ivermectin showing disintegration of plasma membrane with loss of its electron density (arrowhead) and detaching with swollen areas (thick arrow) surrounding intact nucleus (N) in the head pieces of many spermatozoa. Intact acrosomes (thin arrow) are also seen, (x 6000). **Fig. (7):** Ultrathin section in different pieces of spermatozoa of frozen-thawed semen of a bull treated with first dose of ivermectin showing a head piece with normal acrosome (A) and normal nucleus (N). Approximately normal mitochondria (M) and longitudinal fibers (Lf) of a mid piece are also see, (x 8000). **Fig. (8):** Ultrathin section of spermatozoa of frozen-thawed semen of a bull after second dose of ivermectin treatment showing the swelling and separation of plasma membranes surrounding normal nuclei (N) in the head pieces (thin arrow), loose and degeneration of acrosome (arrowhead), distorted mitochondria (thick arrow) in mid piece, and slightly deformation of axoneme (Ax) in tail pieces, (x 6000). **Fig. (9):** Ultrathin section of spermatozoa of frozen-thawed semen of a bull after treatment with a second dose of ivermectin showing intermediate pieces with distorted mitochondrial cristae (arrowheads). Also, swollen plasma membrane (arrow) surrounding intact nucleus (N) in a head piece is seen, (x 6000).

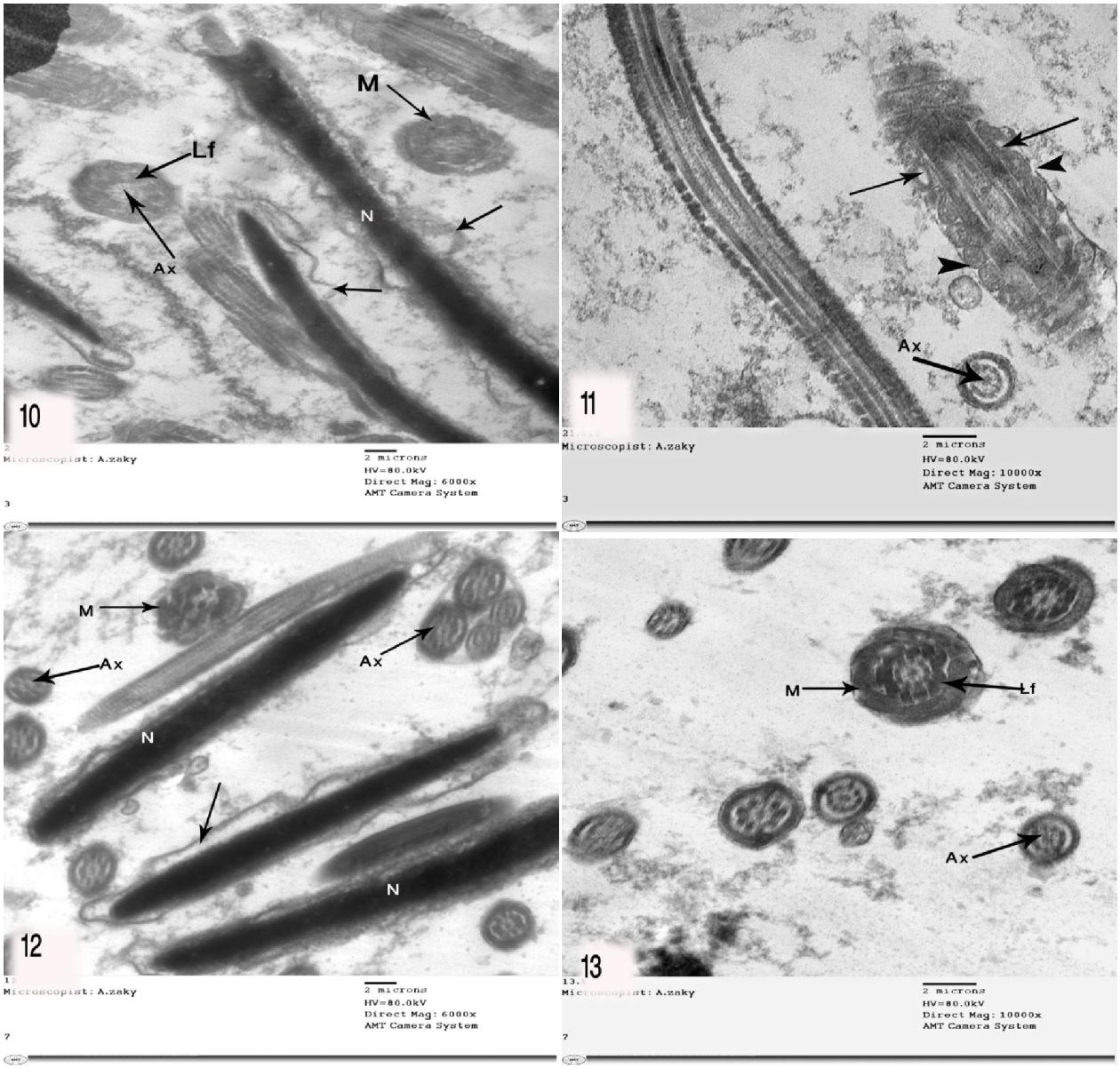

Fig. (10): Ultrathin section of spermatozoa of frozen –thawed semen of a bull treated with the first dose of levamisole showing disintegration and abnormality of plasma membranes with fine dusty materials and detaching with swollen areas (thin arrows) surrounding normal nuclei (N) with homogenous condensed chromatin in the head pieces. Mid pieces are also seen with distorted mitochondria (M), disorganization and distortion of the longitudinal fibers (Lf) and slight deformed axoneme (Ax), (x 6000). **Fig. (11):** Ultrathin section of spermatozoa of frozen –thawed semen of a bull treated with the first dose of levamisole showing distorted mitochondria (arrows) and lysis of plasma membrane (arrowheads) surrounding the mid piece. The end piece is also seen with slight deformed axoneme (Ax), (x 10000). **Fig. (12):** Ultrathin section of spermatozoa of frozen –thawed semen of a bull treated with the second dose of levamisole showing the swelling and separation of plasma membranes surrounding normal nuclei (N) in the head piece (arrow), distorted mitochondria (M) in mid piece, and slightly deformation of axoneme (Ax) in tail pieces, (x 6000). **Fig. (13):** Ultrathin section of spermatozoa of frozen –thawed semen of a bull treated with the second dose of levamisole showing mid pieces with distorted mitochondria (M) and irregular fused longitudinal fibers (Lf), and slightly deformation of axoneme (Ax) in tail pieces, (x 10000).

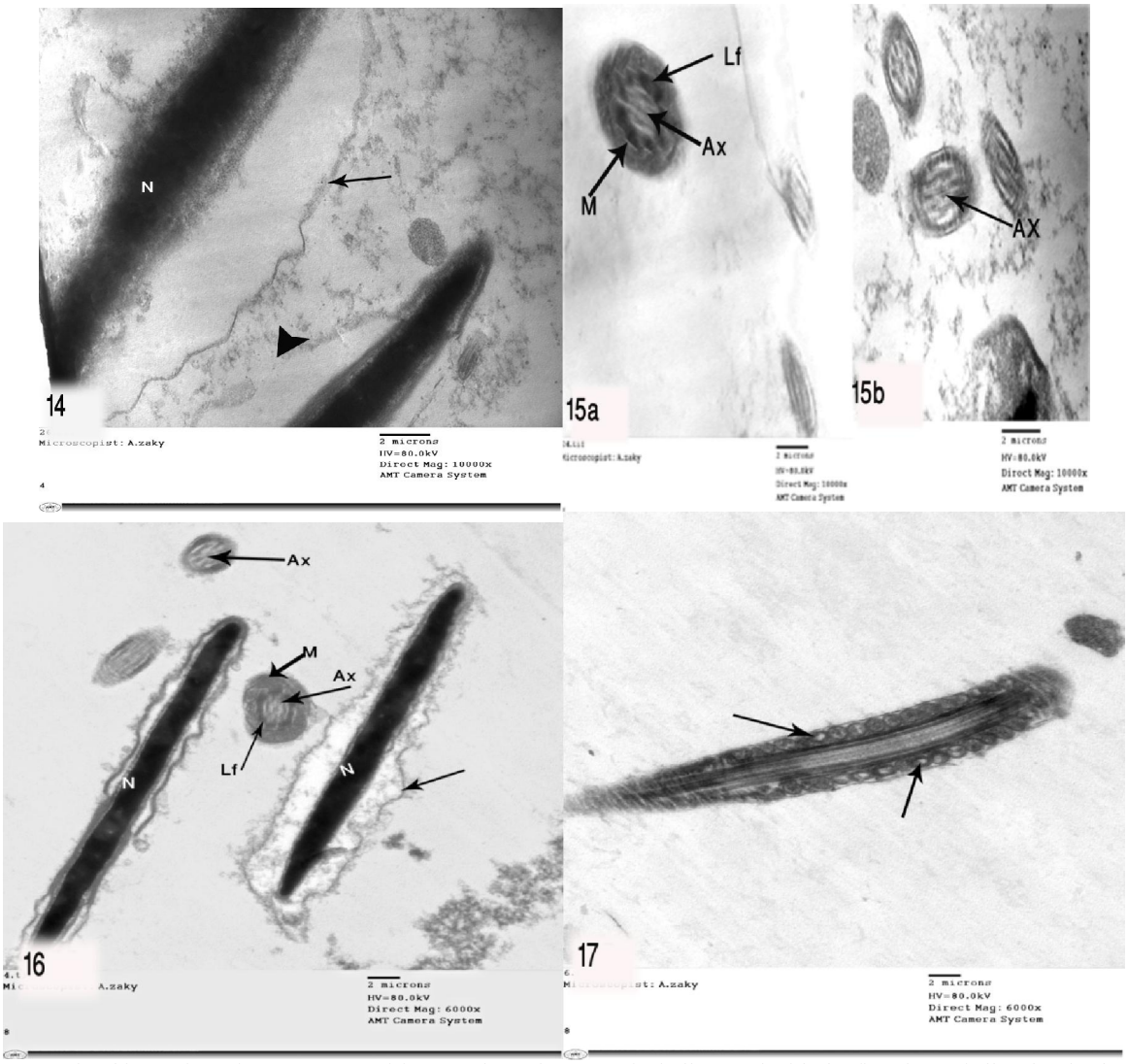

Fig. (14): Ultrathin section of spermatozoa of frozen –thawed semen of a bull treated with the first dose of albendazole showing severe disintegration form and lysis of plasma membrane (arrowhead) and others seen clearly with detaching and swollen (arrow) surrounding abnormal nucleus (N) with unhomogenous condensed chromatin in the head pieces, (x 10000). **Fig. (15a, b):** Ultrathin section of spermatozoa of frozen – thawed semen of a bull treated with the first dose of albendazole showing: a) intermediate piece with distorted mitochondria (M) and irregular fused longitudinal fibers (Lf), and b) completely deformation of axoneme (Ax) in tail pieces (x 10000). **Fig. (16):** Ultrathin section of spermatozoa of frozen–thawed semen of a bull treated with the second dose of albendazole showing severe detaching and swollen of the plasma membrane (arrow) surrounding nucleus (N) in the head pieces. A part of mid piece with distorted mitochondria (M), fused longitudinal fibers (Lf), and terminal tail pieces with deformation and disintegration of axoneme (Ax) are also observed,
(x 6000). **Fig. (17):** Ultrathin section of spermatozoa of frozen–thawed semen of a bull treated with the second dose of albendazole showing the increment of distorted mitochondria (arrows) in a mid piece, (x 6000).

Moreover, severe damage and increment of distorted spermatozoa ultrastructure were seen after the second dose of albendazole in the present work; the spermatozoa showed unhomogeneous chromatin materials in the nuclei, severe form of plasma membrane disintegration, fusion of longitudinal fibers and axonemes, and abnormal appearance of principal and terminal pieces of the tail region.

Ivermcetin is abroad spectrum anti-parasitic agent belonging to the avermactine family, it is highly acceptable anti-nematode and ecto-parasite drug because of its broad range of activity and widely rage of safety. The veterinarian are used as a routine treatment against nematode before and within the breeding seasons. Ivermctin can diffuse to all tissue compartments except the central nervous system after being taken orally or in other ways **(Lankas et al., 1989). El-Nahas and El-Ashmawy (2008)** recorded ivermectin administration once weekly for 8 weeks induced a decrease in testis weights, epididymis and

accessory sex organs and did not change sperm characteristics. But, giving verapamil plus ivermectin significantly induced hazardous effects on sperm count, motility and abnormality**.** However, **Naoman (2012)** did not prefer to use ivermcetin during breeding season due to harmful effect on semen quality and semen testosterone level

Levamisole is highly acceptable anti-nematodal drug and immunomodulatory agent because of its broad range of activity in a large number of hosts. This nematicide targets the nicotinergic acetylcholine receptor, resulting in depolarisation of neuronal and muscle **(Culeuo *et al.*, 2004)**.

The present results showed that post treatment with anthelmintic drugs especially albendazole, the mitochondrial abnormalities were mostly accompanied by defects in the structure of axoneme. Albendazole may interfere with sperm activity by affecting mainly in mitochondria and longitudinal fibers in tail region accompanied by deformation in the motility system indicated by axoneme disorganization and irregularities of tail coarse fibers. This could also directly participate in the reduction of sperm motility.

Morphological and ultrastructural studies of spermatozoa have become an integral part of the modern semen analysis **(Fawcett and Phillips, 1972).** Sperm morphology has been reported as the most important indicator of fertility and has significant prognostic factor in determining the outcome of *in vitro* **(Cancel *et al.*, 2000)** fertilization, outweighing other conventional semen analysis parameters, including sperm- cell concentration and motility, and *in vivo* **(Oyeyymi and Ubiogoro, 2005).**

Axonemal alterations are included the missing of some peripheral microtubules and more commonly missing the central microtubules. These results are in agreement with those of **Miki *et al.* (2004)** and **Saxena *et al.* (2004)** who indicated that glyceraldehyde 3-phosphate dehydrogenase-S, a sperm-specific glycolytic enzyme, is required for sperm motility and male fertility. Although glycolysis is highly conserved, it is remarkable that several unique isozymes in this central metabolic pathway are found in mammalian sperm. It is tightly bound to the fibrous sheath, a cytoskeletal structure that extends most of the length of the sperm flagellum. Moreover, **Dayan (2003)** showed that the albendazole inhibited the polymerization of the parasite tubulin into microtubules. There is a higher affinity of albendazole to the parasite tubulin and so the activity is mediated mainly against the parasites rather than on the host. The loss of the cytoplasmic microtubules leads to impaired uptake of glucose by the larval and adult stages of the parasites.

Similarly, Fenbendazole, is a benzimidazole antihelmintic used in domesticated animals for the treatment and control of nematode parasites, may be affecting sperm mobility by some molecular alteration of the sperm tail axoneme or midpiece, both of which are tubulin containing structures **Bakst *et al.* (2006*).***

Spermatozoa subjected to freezing exhibited plasma membrane loss or damage of the head region. In addition, acrosomes were partially disrupted by freezing and thawing; many acrosomes became swollen, between 50% and 85% of spermatozoa maintained outer acrosomal membrane integrity after freezing **(Holt and North, 1994)**. Also, membrane alterations, mitochondrial distortion, and motility system deformation could be responsible for inducing the increased death rate in incubated spermatozoa**.** The drastic alteration of sperm ultrastructure, particularly of sperm nuclei and mitochondrial sheath became evident mainly after freezing and thawing semen of buffalo and bovine, respectively **(Zeidan *et al*., 1998).**

Moreover, plasma membrane is responsible for the preservation of cellular homeostasis; in this way the plasma membrane integrity exerts a vital role on sperm survival inside the female reproductive tract and on preservation of sperm fertilizing capacity **(Flesch and Gadella, 2000; Andrade *et al.*, 2007).**

Assessment of sperm plasma membrane integrity is one of the key parameters in evaluation of spermatozoal quality in relation to fertility in a particular male. One of the major features discriminating dead from live cells is loss in physical integrity of their plasma membranes and loss of motility **(Burks and Sailing, 1992)**

It was found a positive relationship between increasing seminal plasma total proteins and albumin and increasing total number of sperm output. Seminal plasma alkaline phosphatase (ALP) and acid phosphatase (ACP) These enzymes play a pivotal role in providing substrate energy forming essential link in the energy generating cycles in sperm metabolism, in fertilization process and in the maintenance of constant osmotic pressure during preservation **(Osama and El-Sahn, 2006). El-Seiby *et al*. (2008)** recorded that the high fertile male rabbit strains had phosphatase enzymes activities higher than the low fertile strains. The phosphatase enzymes in semen play an important role in transamination and phosphorylation processes in sperm metabolism and thus explain the differences observed in the semen quality.

Tabl *et al*. (2013) demonstrated that the bulls treated with Ivermectin improved semen qualities by increasing seminal volume and concentration. They recorded an increase in semen- ejaculate volume, sperm- cells concentration, total sperm output ejaculate, ALP and ACP activities in seminal plasma, total proteins, globulin and zinc concentration; while a decrease in seminal plasma alanine-aminotransferase

(ALT), aspartate-aminotranferase (AST), and sodium were reported. The bulls treated with levamisole recorded similar results. While, in contrary, the bulls treated with albendazole recorded opposite results

Mitochondria are in the sheath of the mid - piece of spermatozoa and deliver adenosine triphosphate (ATP) to the axenome where it is utilized for flagellar propulsion. These organelles are required for efficient energy metabolism, production of membrane lipids and cell growth but are also the primary determinants of cellular life or death **(Liu *et al.*, 1996)**. The mitochondrial enzymatic system could be responsible for inducing decrease in spermatozoa motility as mitochondria are concerned with providing energy required for sperm motility **(Rahmy and Ayoub, 2002)**. Moreover, **Selvaraju *et al.* (2011)** suggested that the mitochondrial membrane of spermatozoa primarily affected even with lowest doses of toxic potential of chemicals, and also the decreased mitochondrial membrane potential was associated with sperm abnormalities by the increased reactive oxygen species production **(Wang *et al.*, 2003)**.

It could be recommended from the present work that the treatment of bulls with anthelmintic ivermectin (at a therapeutic dose) for artificial insemination programmes must be used to enhance and keep the highest frozen – thawed sperm motility, freezability, highest intact spermatozoa structure with normal acrosomes, maintained nuclei integrity, mitochondria and axonemes as well as fertilizing efficiency of bull's spermatozoa. In addition, levamisole treatment should be used carefully to the bulls especially during breeding season. Albendazole treatment should be avoided for its harmful effects on ultrastructure of spermatozoa.

References

1. **Al-Qarawi, A. A.; Omar, H. M.; Abdel-Rahman, H. A.; El-Mougy, S. A. and El-Belely, M. S. (2004):** Trypanosomiasis-induced infertility in dromedary *(Camelusdromedarius)* bulls: changes in plasma steroids concentration and semen characteristics. Animal. Reprod. Sci., **84**: 73-82.
2. **Andrade, A. F. C.; Arruda, R. P.; Celeghini, E. C. C.; Nascimento, J.; Martins, S. M.; Raphael, C. F. and Moretti, A. S. (2007):** Fluorescent stain method for the simultaneous determination of mitochondrial potential and integrity of plasma and acrosomal membranes in boar sperm. Reprod. Dom. Anim., **42**:190-194.
3. **Anway, M. D.; Cupp, A. S.; Uzumcu, M. and Skinner, M. K. (2005):** Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science, **308**:1466–1469.
4. **Bakst, R.; Kramer, M. and Long, J. (2006):** Effect of fenbendazole on turkey semen quality. J. Appl. Poult. Res., **15**:307–311.
5. **Benz. G. W.; Roncalli, R. A. and Gross, S.J. (1989):** Use of Ivermectin in Cattle, Sheep, Goats and Swine. In: Cambell WC, ed. Ivermectin and Abamectin. New York: Springer Verlag. PP: 214-234.
6. **Burks, D.J. and Sailing, P. M. (1992):** Molecular mechanisms of fertilization and activation of development. Anim. Reprod. Sci., **28**:79-86.
7. **Cancel, A.; Lobdell, D.; Mendola, P. and Perreault, S. (2000):** Objective evaluation of hyperactivated motility in rat spermatozoa using computer-assisted sperm analysis. Hum. Reprod., **15**: 1322-1328.
8. **Corderodel C. M. (1999):** Parasitologia Veterinaria. McGraw-Hill- Interamericana de Espana, S.A.U.; Madrid.
9. **Culeuo, E.; Baylis, H. A.; Richmond, J. E.; Jones, A. K. and Fleming, J. T. (2004):** The Caenorhabditiselegans unc-63 gene encodes a Levamisole-sensitive nicotinic acetylcholine receptor alpha subunit. J. Biol. Chem., **279**: 42476–42483.
10. **Dayan, A. D. (2003):** Albendazole, mebendazole and praziquantel. Review of non-clinical toxicity and pharmacokinetics. Acta Trop., **86**:141-159.
11. **El-Nahas, A. F. and El-Ashmawy, I. M. (2008):** Effect of ivermectin on male fertility and its interaction with P-glycoprotein inhibitor (verapamil) in rats. Environ. Toxicol. Pharmacol., **26**: 206–211.
12. **El-Seiby, M. E.; Elkomy, A.E. and Kamel, K.I. (2008):** Evaluation of spermatological parameters and free amino acids composition of Black Baladi, New Zealand White andV-Line rabbits buck under winter Egyptian condition. Egypt. Poult. Sci., **28**: 617-631
13. **Fawcett, D. W. and Phillips, D. M. (1972):** Recent Observations on the Ultra-Structure of Mammalian Spermatozoa. In: Comparative Spermatology. B. Bacetti (Ed). Academic Press, New York. PP: 2- 46.
14. **Fleming, J. T.; Squire, M. D, Barnes, T. M.; Tornoe, C. and Matsuda, K. (1997):** Caenorhabditiseleganslevamisole resistance genes lev-1, unc-29, and unc-38 encode functional nicotinic acetylcholine receptor subunits. J. Neurosci., **17**: 5843–5857.
15. **Flesch, F. M. and Gadella, B. M. (2000):** Dynamics of the mammalian sperm membrane in the proces of fertilization. Biochem.Biophys.Acta, **1469**:197-235.
16. **Folador, A. (2000):** Parameters evaluation and determination of reproductiveswistar rats

(*musnorwergicusalbinus*) under avermectins treatment. Scientia Agraria, **1** : 1

17. **Holt, W. V. and North, R. D. (1994):** Effects of temperature and restoration of osmotic equilibrium during thawing on the induction of plasma membrane damage in cryopreserved ram spermatozoa. Biol. Reprod., **51**: 414.
18. **Lankas, G. R.; Minsker, D. H. and Robertson, R. T. (1989):** Effect of ivermectin on reproduction and neonatal toxicity in rats. Food Chem. Toxicol., **48**: 523- 529.
19. **Liu, X.; Kim, C. N. and Yang, J. (1996):** Induction of apoptotic program in cell-free extracts: Requirements for ATP and cytochrome C. Cell, **86**: 147–157.
20. **Mcdowell, E. M. and Trump, B. F. (1976):** Histologic fixatives suitable for diagnostic light and electron microscopy. Arch. Pathol. Lab. Med., **100**: 405-413.
21. **Miki K, W.; Qu, Goulding, E.H.; Willis,W.D.; Bunch, D.O.; Strader, L.F.; Perreault, S.D.; Eddy, E.M. and O'Brien, D.A. (2004):** Glyceraldehyde 3-phosphate dehydrogenase-S, a sperm-specific glycollytic enzyme, is required for sperm motility and male fertility. Proc. Natl. Acad. Sci., **101**:16501-16506.
22. **Mottier, M. L.; Alvarez, L. I.; Pis, M. A. and Lanusse, C. E. (2003):** Tran-stegumental diffusion of benzimidazoleanthelmintics into Monieziabenedeni: correlation with their octanol-water partition coefficients. Exp. Parasitol., **103**: 1-7.
23. **Naoman, U. D. (2012):** Effect of Ivermectin on semen characteristics of Iraqi Awassi ram**.** Al-Anbar J. Vet. Sci., **5** (2): 129-133
24. **Osama, M. Aly and El-Sahn, A. (2006):** Effect of crossing on the performance of local strains. 3. Seminal quality, electrophoretic pattern of seminal plasma proteins, fertility and hatchability in Bandara, Gimmizah and their reciprocal crosses. Egypt. Poul. Sci., **26**: 123-136.
25. **Oyeyemi, M. O. and Ubiogoro, O. (2005):** Spermiogram and morphological characteristics in testicular and epididymal spermatozoa of large boar in Nigeria. Int. J. Morphol., **23**: 235-239. **Rahmy, T. R. and Ayoub, M. A. (2002):** *In vitro* action of cobra venom on goat spermatozoa ultrastructure by transmission and scanning electron microscopy. J. Venom. Anim. Toxins, **8**:127- 159.
26. **Raisinghani, P. M. (1992):** Helminthic diseases of the dromedary camels in India. In: Proceedings of the 1st International Camel Conference, United Arab Emirates, 105-106.
27. **Reynolds, E.S. (1963):** The use of lead nitrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol., **17**: 208.
28. **Salisbury, G. W.; Van Denmark, N. L. and Lodge, J. R. (1978):** Physiology of Reproduction and AI of Cattle. W. N. Freeman and Co. San Fransisco, USA.
29. **Saxena, A. K.; Dhungel, S.; Bhattacharya, S.; Jha, C. B. and Srivastava, A. K. (2004):** Effect of chronic low dose of methotrexate on cellular proliferation during spermatogenesis in rats. Arch. Androl., **50**:33-35.
30. **Selvaraju, S.;Nandi, S.; Gupta, P. and Ravindra, J. (2011):** Effects of heavy metals and pesticides on buffalo (*Bubalusbubalis*) spermatozoa functions *in vitro*.Reprod. Domest. Anim., 46: 807-813.
31. **Tabl, G. A.; El-Desouki I.N.; Zeidan, A.E; Tag El- Deen, M. A and Kamel, R. (2013):** Effect of Anthelmintic Drugs on Biochemical Characteristics of Bull Friesian Frozen Semen. Proc. 7Th Int. Con. Biol. Sci. In press
32. **Tag El-Dein, M. A.; Zeidan, A. E. B.; El-Desouki, N. I.; Tabl, G. A. and Kamel, R. A. (2011):** Freezability, DNA integrity and fertilizing capacity of spermatozoa of Friezian bulls treatment with anti-helminthic drugs. J. Anim. Poult. Prod., **2** (8): 311- 326.
33. **Tanyildizi, S. and Bozkurt, T. (2002):** An investigation of the effects of ivermectin on blood serum, semen hyaluronidase activities and spermatological characteristics in sheep.Turk. J. Vet. Anim. Sci., **26**: 353-357.
34. **Teruel, M.; Garcia, V. and Catalano, R. (2009):** Effect of albendazole sulphide on embryo,foetal and placental parameters in Wister rats.Int. J. Morphol., 27: 1147- 1153
35. **Wang, X.; Sharma, R. K.; Gupta, A.; George, V.; Thomas, A. J.; Falcone, T. and Agarwal, A. (2003):** Alterations in mitochondria membrane potential and oxidative stress in infertile men: a prospective observational study. Fertil.Steril.,**2**: 844-850.
36. **Zeidan, A. E. B.; El-Gaafary, M. N. and El-Keraby, F. E. (1998):** Effect of new packaging methods for frozen-bull semen in pellets form on some biochemical changes and conception rate.Proc. 1st Intern.Conf.Anim. Prod. and Health in Semi-Arid Areas, El-Arish, North Sinai, Egypt, pp: 223-234.