EFFECT OF ANTHELMINTICS DRUGS ON BIOCHEMICAL CHARACTERISTICS OF BULL FRIESIAN FROZEN SEMEN

Ghada, A. Tabl *; Nabila, I. El-Desouki*; Alaa, E. B. Zeidan**; Mohamed, A. Tag El-Deen** and Rabab, A. Kamel***

* Department of Zoology, Faculty of Science, Tanta University, Egypt

** Animal Production Research Institute, Dokki, Giza, Egypt

*** Department of Physics, Faculty of Science and Arts in Al-Ardah, Gazan University, Kingdom of Saudi Arabia.

ABSTRACT

Three different antihelmintic drugs were used in the present study (ivermectin, levamisole and albendazole) on Friesian bulls .The animals were divided into four groups (control untreated group, ivermectin, levamisole, and albendazole treated groups, respectively). The drugs used at two doses with 8 weeks intervals between first and second one at 200µg/kg, 7.5mg/kg and 10mg/kg, respectively. Semen samples were collected for 8th weeks after each dose. Biochemical semen characteristics were estimated. The bulls treated with ivermectin showed significant increase in semen- ejaculate volume, sperm- cells concentration ($x10^9$ / ml), total sperm output ejaculate ($x10^9$ / ml), alkaline phosphatase (ALP) and prostatic acid phosphatase (ACP) activities in seminal plasma, seminal plasma total proteins, globulin, urea and zinc concentration; while significantly decreased seminal plasma alanine-aminotransferase (ALT), aspartate-aminotranferase (AST), and sodium were reported. The bulls treated with levamisole showed significant increase in semen-ejaculate volume, total sperm output/ejaculate, ALP and prostatic ACP activities in seminal plasma, seminal plasma total proteins, globulin, urea and zinc concentrations; while significantly decreased seminal plasma ALT, AST, and sodium were recorded. In contrary, the bulls treated with albendazole were significantly decreased semen-ejaculate volume, sperm motility, seminal plasma total proteins, globulin, creatinine and calcium. Also, it showed significant increase in seminal plasma enzymes activity (ALT, AST and ALP) and zinc concentrations. It is interest to note that different anthelmintics treatment used in this study didn't affect significantly in seminal plasma albumin and total ACP activity. In conclusion: Ivermectin treatment improved semen qualities by increasing seminal volume and concentration. Anthelmintics treatment with levamisole should be used carefully under control during breeding season. Albendazole treatment should be avoided because its harmful effect in semen by decreasing semen-ejaculate volume, sperm motility, seminal plasma total proteins, globulin and increasing seminal plasma aminotransferase enzymes activity.

Key Words: Anthelmintics, Bulls, Frozen Semen, Physical & Chemical estimation

INTRODUCTION

The male sexual functions are very sensitive to pharmacological agents. Male reproductive function is known to be highly sensitive to many chemicals and physical agents generated by industrial or agricultural activities **(Bonde, 1996**; **Spira and Multigner, 1998; Favareto *et al.*, 2011)**. Numerous investigations were carried out to detect the effect of drugs on male sexual functions, different chemical classes of Pesticides and solvents have been demonstrated to be male reproductive toxicants in animal models **(Sundaram and Witorsch, 1995).**

The environmental risks of pharmaceuticals have been studied less frequently in comparison to other chemicals such as pesticides and biocides. Nevertheless, during the last few years, veterinary and human medicinal products gained increasingly more attention. A wide range of medicinal products used in veterinary medicine include various groups of chemicals, purposes for companion and farm animals. These drugs may have beneficial or deleterious effects on the fertility of the animals **(Abdulhakeem *et al.*, 2006)**. The parasiticides and antibiotics are two of the most important groups and as such used fairly often in animal treatment. There are different entry routes of veterinary drugs into the environment. Manure of treated farm animals may contain significant amounts of the active ingredients or metabolites. They can be excreted from treated animals in agricultural soils directly (pasture) or with the application of manure as a fertilizer**(Kolar and Kozuh, 2006).**

Research findings have shown that both synthetic and natural drugs have considerable effects on the male reproductive

system, especially the spermatozoa of domestic animals and man **(Etta *et al.*, 2009)**. In veterinary practice, several chemotherapeutic and other chemical agents are administered to animals to treat some infectious diseases and/or to achieve predetermined physiological modifications such as anesthesia or smooth muscle contraction amongst others **(Benson, 2000; Abdulhakeem *et al.*, 2006)**. Also, **Sukumaran *et al.* (2010)** showed that some antiepileptic drugs, such as phenobarbital and carbamazepine, are powerful inducers of hepatic enzymes that are involved in the metabolism of sex hormones. Antiepileptic drugs with hepatic enzyme induction property may alter the blood level of progesterone, estrogen and other sexual hormones and may potentially interfere with reproductive functions.

To ensure the safety and to evaluate possible, male reproductive health - related problems associated with the use of anthelmintic drugs, the present investigation was planned to study the effect of three different drugs (ivermectin, levamisole and albendazole) on physical and chemical semen characteristics of Friesian bulls or after invasion (Sam-Yellowe, 1992; Rungruang *et al*., 2005) and participating in the formation of the parasitophorous vacuolar membrane (Ling *et al*., 2003; 2004), the exact function of the complex has not been determined yet.

In mammalian cells, sorting of transmembrane proteins is mediated by cytoplasmic adaptor complexes (APs) which recognize specific motifs (e.g. the YXXø motif, where ø is a hydrophobic amino acid) within their cytoplasmic tails. APs select cargo for inclusion into a transport vesicle and recruit coat components (e.g. clathrin) necessary for vesicular budding and transport (Robinson, 2004; Bonifacino and Lippincott-Schwartz J., 2003). This mechanism has been shown to operate in *Toxoplasma*, and may also be conserved in *Plasmodium* (Hoppe *et al*., 2000; Bhanot *et al*., 2003). However, most *Plasmodium* rhoptry proteins described to date do not possess a transmembrane region and cytoplasmic tail, implying the existence of additional sorting pathways (Kats *et al*., 2006). One possibility is that sorting within the Golgi occurs via a clustering mechanism whereby proteins en route to a particular destination aggregate into distinct sub-domains like what happens in mammalian cells (Glombik and *Gerdes*, 2000).

Proteins targeted to the apicoplast share general features and use common targeting signals (Foth *et al*., 2003) and so the proteins are exported to the host erythrocyte (Hiller *et al*., 2004; Marti *et al*., 2004). However, rhoptry proteins do not have a yet identified common signal to target them to their destination. Since rhoptry proteins are important in the formation of the parasitophorous vacuoles and in the invasion of host cell, the accurate targeting of these proteins to their final destination must be an essential process for the invasion of erythrocytes and the growth of parasites.

The rhoptry associated membrane antigen (RAMA) is a glycosylphosphatidyl inositol-anchored protein that is synthesized and expressed first in the late ring stage before the appearance of recognizable rhoptries, and appears to temporarily accumulate within compartments of the secretory pathway (Topolska *et al*., 2004). Fluorescence Resonance Energy Transfer experiments indicated that RAMA interacts with the low molecular weight (LMW) rhoptry complex (Topolska *et al*., 2004). Rhoptry targeting of the LMW complex (heterodimer composed of RAP1, and RAP2 or RAP3) occurs via the N-terminus of RAP1, although the mechanism is not understood (Baldi *et al*., 2000). RAMA has been hypothesized to act as an escorter for RAP1 to recruit RAP1, RAP-2 and RAP-3 into a rhoptry-destined protein complex.

Ghoneim *et al*. (2007) determined that the N-terminal 24 amino acids of RhopH2, including signal peptide sequence, are sufficient to target GFP to the rhoptries under the control of *rhoph2* promoter and proposed that this targeting is likely mediated by a unique mechanism that depends on the interaction with N-terminal 24 amino acids of RhopH2 early in the secretory pathway. This targeting has been shown to be sensitive to Brefeldin A and thus implied that the 5 amino acids downstream of the signal peptide cleavage site may contain the sorting signal required for rhoptry targeting (Ghoneim, in press). The present study tried to determine whether Clag3.1, a member of RhopH complex, is trafficked to rhoptries under the same control elements or rather uses an alternative targeting mechanism. To do this, a new transgenic *Plasmodium falciparum* line expressing ¾ of Clag3.1 fused to the N-terminus of GFP under the control of RhopH2 promoter was established and the expression the GFP chimera was monitored in live parasites.

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 Delata (31°N), belonging to Animal Production Research Institute, Agricultural Research Center, Egypt during the period from May,2009 to April, 2010.

Animals and Drugs

Sixteen Friesian bulls with an initial live body weight of 380±10.2 kg and aged 20 months were used in the present study. All bulls were healthy and clinically free of external and internal parasites. Palpation of the external genetalia tract showed that they were typically normal. The testicular tone was glandular, all epididymal regions were present and both testes were almost equal in size and moved freely up and down within the scrotal puches. Copulatory patterns for all tested bulls, at the beginning of the experiment were judged to be normal. Bulls were randomly divided into four groups (4 each). Bulls in the first group were untreated acting as control, while those in the second, third and fourth groups were treated with ivermectin (200µg/kg, subcutaneously), levamisole HCl (7.5mg/kg, subcutaneously) and albendazole (10mg/kg, orally), respectively, as a first dose. The second dose repetition was administered after 8 weeks from the first dose. Ivermectin (Paramectin) and levamisole HCl (Levapan 10%) were supplied by Pharma Swede- Egypt and albendazole (Delta zole 10%) was supplied by Delta Pharma, Egypt.

Experimental procedures:

Semen samples were collected from the all bulls of experimental groups biweekly 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). Semen was collected in collecting tube. Semen-ejaculate volume, percentage of progressive motility, live sperm and sperm abnormalities, sperm-cells concentration ($x10^9$/ml) and total-sperm output ($x10^9$/ml) were estimated for each ejaculate according to **Salisbury *et al.* (1978)**. Semen samples were centrifuged at 600g for 20 minutes. The supernatant of seminal plasma was removed and stored at -20°C till biochemically analysis. Aspartate-aminotransferase (AST) and alanin-aminotransferase (ALT) were determined as described by **Tietz** (**1995**) using Roche Biological kits. Acid phosphatase (ACP) was also determined as described by **Kind and King (1954)** by using colorimetric method. Alkaline

phosphatase (ALP) was determined by **Guder (1996)** using Roch kits. Total proteins concentration was determined colourimetrically according to Biuret method as described by **Weichselbaum (1946)**. Albumine concentration was determined colourimetrically by bromocresol green method according to **Doumas *et al*. (1971)**, globulin concentration was calculated by subtraction albumin from the total proteins contents. Urea and creatinine concentrations were determined colourimetrically by **Jung and Parek (1970)** and **Bartels *et al.* (1972),** respectively. Sodium and calcium concentrations were determined colourimetically according to the method described by **Trinder (1951)** and **Gindler (1972),** respectively. Zinc concentration was also determined using 5pg Atomic Absorption Spectrophotometry (Pye Unicam) according to the method of **Willis (1960)**.

All enzymatic activities in the seminal plasma (ALT, AST, ALP and ACP) were adjusted according to sperm-cell concentration ($U/10^9$ spermatozoa) according to **Reitman and Frankle (1957).**

***Statistical analysis*:**

Data were presented as the mean ± SE, and significant differences were determined by Duncan's multiple range tests **(Duncan, 1955)** using SPSS statistical analysis software. P values less than 0.05 were considered significant.

RESULTS

1. Semen characteristics:

Data presented in Table (1)reported that after the first dose of the treatment of ivermectin or livamesole to the bulls, significant (P<0.05) increase in semen-ejaculate volume, percentage of live spermatozoa, sperm-cells concentration and total-sperm output. After the second dose, significant (P<0.05) decrease in sperm abnormalities as compared to untreated bulls was recorded. The bulls treated with first and second dose of albendazole showed significantly (P<0.05) decrease in semen-ejaculate volume and sperm motility as compared to the other experimental bulls.

2. Biochemical semen characteristics:

2.1. Enzymatic activity:

The results presents in Table (2) revealed that with the first dose, the bulls treated with ivermectin recorded significantly (P<0.05) increase in alanine aminotransferase (ALT), alkaline phophatase (ALP) and prostatic acid phosphatase (prost ACP) activities. The bulls treated with levamisole showed significantly (P<0.05) decrease in ALT and aspartate-aminotransferase (AST) and significant (P<0.05) increase in ALP and ProsACP activities. The bulls treated with albendazole showed significantly (P<0.05) increase in ALT, AST, and ALP activities as compared to untreated bulls.

Regard to the second dose, bulls treated with ivermectin showed significantly (P<0.05) increase in ALP and decrease in ALT, AST and prostatic ACP activities. The bulls treated with levamisole showed significantly (P<0.05) increase in ALT, ALP and prostACP. The bulls treated with albendazole showed significantly (P<0.05) increase in ALT and ALP activities and significant decrease in prostatic ACP activity.

Total acid phosphatase (ACP total) activity didn't affected with anthelmintics treatment either first or second dose.

2.2. Seminal plasma constituents:

Data represented in Table (3) showed that the seminal plasma total proteins, globulin and zinc of the bulls treated with the first dose of ivremectin was significantly (P<0.05) increased, while seminal plasma sodium was significantly (P<0.05) decreased. Also, the first dose of levamisole treatment showed significant (P<0.05) increase in seminal plasma total proteins and zinc and significant decrease in sodium concentration as compared to untreated bulls. In contrary, the bulls treated with first dose of albendazole showed significant (P<0.05) decrease in seminal plasma total proteins, globulin, creatinine, calcium, and significant (P<0.05) increase in sodium and zinc concentrations.

Regarding to second dose, the bulls treated with ivermectin were significantly (P<0.05) increase in seminal plasma total proteins, globulin and urea, and significantly (P<0.05) decrease in sodium and zinc concentrations as compared to untreated bulls. Also, the bulls treated with levamisole showed significantly (P<0.05) increase in seminal plasma total proteins, globulin and urea. The bulls treated with albendazole showed significantly (P<0.05) increase in globulin, urea and zinc and significantly (P<0.05) decrease in sodium concentration.

Anthelmintics treatment of ivermectin or levamisole caused insignificant increase in seminal plasma albumin concentration and albendazole treated bulls showed insignificant decrease in albumin concentration

Discussion

The present results recorded that the treatment of ivermectin or livamesole to the bulls recorded significant increase in semen-ejaculate volume, percentage of live spermatozoa, sperm-cells concentration and total-sperm output. In accordance, **Tanyildizi and Bozkurt (2002)** reported that ivermectin increased semen-ejaculate volume in rams (P<0.01) in comparison with the control group. Also, **Mejia *et al.* (1999)** demonstrated that continuous ivermectin treatment in dairy heifers grown on nematode-infected pastures can increase growth rate and advance the onset of ovarian function, and affects the yearling heifer's pelvic area. Moreover, **Janett *et al.* (2001)** reported that stallions treated with Eqvalan had significantly better concentration and motility and viability of sperm and regarding morphology, normal sperm, major defects and vacuoles were significantly better in the Eqvalan group.

In contrary, **Onakpa *et al.* (2010)** reported that diminazene aceturate and ivermectin decreased semen parameters and serum testosterone and follicle stimulating hormone in red Sokoto bucks. Similar results using diminazene aceturate were demonstrated in ram **(Tanyildizi and Turk, 2004)** and ivermectin in sheep **(Tanyildizi and Bozkurt, 2002)**.Moreover, many authors recorded the undesirable effects of ivermectin on male fertility, as reduced semen concentration and sperm motility **(Schroder *et al.*, 1986).**

Many reports found that the decrease in reproductive organs weight that caused by ivermectin could be due to the decrease in testosterone level **(El-Ashmawy and Mandour, 1996; Srikhanth *et al.*, 1999; Zaied, 2004)**, which may be due to the direct effect of ivermectin on the central nervous system and gonadal tissues or its effects on hypothalamus–pituitary–testis axis **(El-Nahas and El-Ashmawy, 2008).**

Ivermectin when adminesterd with verapamil reduced sperm content which implies an adverse effect on spermatogenesis in rats, impaired sperm motility in these rats is indicative of a defect in the acquisition or maintenance of sperm motility. These combinations may alter the epididymal secretory products or has a direct action on sperm motility and morphology **(Ballent *et al.*, 2007; Zaied, 2004).**

Sukru *et al.* (2007) showed that the use of levamisole as an immunomodulator should not be considered during the breeding season due to lower pregnancy rate. The disruption of the establishment of pregnancy by immunomodulatory treatment with levamisole was seen possibly via activation of general, as well as, uterine immune system. Also, it has been suggested that the use of levamisole should be avoided during the breeding season in rams, because of a harmful effect on semen's quality **(Bozkurt *et al.*, 2004).** Moreover, **Kazy *et al.* (2004)** postulated that the orally administered dose of levamisole used before or during the entire pregnancy in rats, decreased implantation and some growth retardation.

The present findings on the undesirable effect of albendazole recorded significant decrease in semen-ejaculate volume and sperm motility as compared to the other experimental bulls. In agreement, **Bakst *et al.* (2006)** showed albendzole affects sperm motility. Moreover, albendazole may be genotoxic to human lymphocytes *in vivo* **(Ozats *et al.*, 2007).** Also, the results of the rat teratology studies showed that high doses of albendazole oxide were embryotoxic and that lower dose (7mg/kg/day) caused impairment of foetal development **(EMEA, 1999).** Reproductive effects of albendazole (5.8 mg/kg bw/day) were investigated in multigeneration oral-dosing studies in rats; it caused reduced survival and growth of pups during the post-natal lactation period **(EMEA, 2004).**

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 8 weeks interval.

In contrary, some investigations postulated that albendazole didn't affect reproductive performance, where, single oral dose of albendazole (22.5mg/kg) didn't alter reproductive functions in bulls **(Berndtson *et al.*, 1980).** Fenbendazole has been shown to have no adverse effects on semen quality or fertility in cattle, sheep, or swine **(Campbell and Rew, 1986).** This is in disagreement with the current results that may be attributed to the species or breed variation of animals used and also to the dosage of drugs administered. Climatic variations may also be a factor responsible for the differences in findings.

The present work showed that the antiparasitic drugs used in this study didn't negatively influence total acid phosphatase (ACP) activity on seminal plasma.The prostatic ACP and alkaline phosphatase (ALP) in seminal plasma showed highest activity after ivermectin, levamisole or albendazole treatment. Also, the alanine-aminotransferase (ALT) and aspartate-aminotranferase (AST) showed lower activity after second dose treatment with ivermectin. In contrary, first or second dose treatment with albendazole showed highest activities of ALT and AST.

The transaminases activities (AST and ALT) in semen are a good indicator of semen quality because it measures sperm membrane stability **(Zedda *et al.*, 1996). Boehnke *et al.* (1978**) found that among 22 different criteria used for evaluating heat stressed bulls, the least useful method were the determination of AST activity. Also, the increments of the activities of AST and ALT in seminal plasma are mainly due to the leakage of these enzymes **(Navarro *et al.*, 1993). Yousef and Zeitoun (1998)** found that there were negative correlation coefficients between decrease sperm motility on one side and AST and ALT release on the other side. They reported that the activities of these enzymes could be used as an indicator of sperm integrity. **Yousef *et al.* (2003)** reported that there was a negative correlation between increased ALT and AST activities and decrease sperm-ejaculate volume, sperm cells concentration, total- sperm output, sperm motility index and total motile sperm. Therefore, the decrease in the activities of these enzymes coincided with the increase of semen quality.

Seminal plasma ALP and ACP 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 **(Dhami and Kodagali, 1987).** 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 **(Dhami *et al.*, 1994).** Also, **Kamel (2005)** found that, in rabbit groups, which have higher seminal quality index the ACP activity increased in their seminal plasma. Moreover, **El-Seiby *et al.* (2008)** found that the high fertile male rabbit strains had phosphatase enzymes activities higher than the low fertile strains. Similar results were found between the high and low fertile male rabbits under the same strain **(Elkomy *et al.*, 2008)**.

In the present work, seminal plasma components in the present study, total proteins, globulin and zinc showed highest concentration after the first and second doses treatment with ivermectin or levamisole, while sodium concentration showed lowest value. First dose of albendazole showed lowest concentration of total proteins, globulin, creatinine, calcium and highest concentration of sodium and zinc, while second dose showed highest concentration of zinc, globulin, urea and lowest sodium value. It is interest to note that different anthelmintics treatment didn't significantly influence albumin concentration.

It is known that seminal plasma proteins coat and protect spermatozoa during ejaculation. **Reson *et al.* (1958)** reported that increase transaminases activities were related to amino acid imbalance that initiates protein catabolism. Many studies have shown that low content of seminal plasma proteins is associated with poor semen quality **(White *et al.*, 1987**; **Ashworth *et al.*, 1994). Kulkarni *et al.* (1996)** showed that, seminal plasma total protein is mainly composed of albumin and globulin, in addition to small quantities of nonprotein nitrogen, amino acids and peptides. These compounds make up the amphoteric property of seminal plasma proteins, thus, low protein content in seminal plasma reduce its buffering capacity and in turn semen quality **(Dhami *et al.*, 1994). Taha *et al.* (2000)** revealed that there was a positive relationship between semen quality and level of seminal plasma total proteins. Similar results were presented by **Osama and El-Sahn (2006)** who found a positive relationship between increasing seminal plasma total proteins and albumin and increasing total number of sperm output. A positive relationship between increase seminal plasma total protein, globulin concentration and increase semen quality was in agreement with **Elkomy *et al.* (2008)** who reported that an increase in seminal plasma total protein and albumin concentrations were showed in high fertile male compared to low fertile rabbits and this increase was associated with increase their seminal quality measurements.

Abdel-Rahman *et al.* (2000) reported that in small rumunants the percentage of motile spermatozoa decreases as the content of potassium and calcium increases and sodium, chloride, phosphorus and magnesium decreases. This is in line with **Bearden and Fuquay (1997)** who reported high concentrations of potassium and calcium to be detrimental to semen metabolism of the bull spermatozoa, which determines the motility of spermatozoa. On the other hand, **Abdel-Rahman *et al.* (2000)** also reported the percentage of live spermatozoa to be correlated positively with potassium and calcium and negatively correlated with phosphorus.

A clear inhibitory effect of free extracellular zinc on the motility of human spermatozoa was demonstrated by previous *in vitro* studies **(Lindholmer, 1974; Riffo *et al.*, 1992)** which were agree with our results which demonstrated that albendazole treatment decreased sperm motility and increased zinc concentration in seminal plasma. **Betteger and O´ Dell (1981)** showed that zinc toxicity on cells might even be mediated by interference by the ion on plasma membrane permeability.

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 8 weeks interval.

In conclusion, the present results reported that the ivermectin treatment improved semen qualities by increasing seminal volume and concentration. Anthelmintics treatment with levamisole should be used carefully under control during breeding season. Albendazole treatment should be avoided because its harmful effect in semen by decreasing semen-ejaculate volume, sperm motility, seminal plasma total proteins, globulin and increasing seminal plasma aminotransferase enzymes activity

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Table(1): Effects of the different anthelmintics (ivermectin, levamisole and albendazole) administration at the first and second doses on some semen characteristics of Friesian bulls.

Item	No. of dose	Treatments				Overall mean
		Control	IVM	LEV	ABZ	
Semen-ejaculate volume (ml)	1	1.95 ± 0.29^{b}	3.1 ± 0.39^{a}	2.86 ± 0.37^{a}	1.29 ± 0.12^{bB}	2.30±0.11
	2	2.3 ± 0.24^{a}	2.52 ± 0.21^{a}	2.35 ± 0.13^{a}	1.76 ± 0.13^{bA}	2.23±0.12
Mean		2.12 ± 0.19^{b}	2.81 ± 0.22^{a}	2.61 ± 0.19^{a}	1.52 ± 0.09^{c}	2.26
Sperm motility (%)	1	74.52 ± 1.91^{a}	79.52 ± 1.68^{a}	76.67 ± 2.24^{a}	67.86 ± 2.61^{b}	74.64±1.21
	2	73.81 ± 1.61^{a}	78.83 ± 1.43^{a}	78.81 ± 1.65^{a}	67.86 ± 2.77^{b}	74.83±1.30
Mean		74.17 ± 1.24^{b}	79.17 ± 1.09^{a}	77.74 ± 1.38^{ab}	67.86 ± 1.88^{c}	74.73
Live spermatozoa (%)	1	80.65 ± 2.17^{b}	86.05 ± 2.74^{a}	85.24 ± 2.43^{a}	76.96 ± 2.95^{b}	82.22±1.12
	2	81.60±2.54	83.77±1.98	82.16±2.5	80.60±1.86	82.03±1.72
Mean		81.12 ± 1.65^{ab}	84.91 ± 1.67^{a}	83.70 ± 1.74^{a}	78.78 ± 1.75^{b}	82.12
Sperm abnormalities (%)	1	7.31 ± 1.19^{B}	8.48±1.51	7.51±1.19	6.54±1.33	7.46 ± 1.40^{B}
	2	11.53 ± 1.74^{Aa}	8.48 ± 1.08^{b}	6.62 ± 1.27^{c}	8.47 ± 1.11^{b}	8.77 ± 0.47^{A}
Mean		9.42±1.09	8.48±0.92	7.07±0.86	7.50±0.87	8.11
Sperm-cells concentration($x10^{9}$/ml)	1	0.96 ± 0.09^{b}	1.44 ± 0.12^{a}	1.22 ± 0.15^{a}	0.89 ± 0.14^{b}	1.13±0.01
	2	1.18 ± 0.12^{a}	1.23 ± 0.14^{a}	1.27 ± 0.13^{a}	0.82 ± 0.13^{b}	1.12±0.14
Mean		1.07 ± 0.08^{ab}	1.34 ± 0.09^{a}	1.25 ± 0.1^{a}	0.85 ± 0.63^{b}	1.13
Total-sperm output ($x10^{9}$/ejaculate)	1	1.83 ± 0.38^{b}	4.64 ± 0.79^{a}	4.13 ± 0.93^{a}	1.23 ± 0.67^{b}	2.96±0.41
	2	2.64 ± 0.36^{ab}	3.21 ± 0.53^{a}	3.12 ± 0.41^{a}	1.62 ± 0.35^{b}	2.65±0.35
Mean		2.24 ± 0.27^{b}	3.93 ± 0.48^{a}	3.62 ± 0.5^{a}	1.42 ± 0.22^{b}	2.80

Control: untreated bulls, IVM: Ivermectin, LEV: Levamisole and ABZ: Albendazole.

a, b and c: Means in the same row with different superscripts differ significantly ($P<0.05$).

A, B: Means in the same column with different superscripts differ significantly

Data were represented by means ± SE.

Table (2): Effects of the different anthelmintics (ivermectin, levamisole and albendazole) administration with the first and second doses on enzymatic activity of Friesian bulls.

Item	No. of Dose	Treatment				Overall mean
		Control	IVM	LEV	ABZ	
ALT ($U/10^9$spermatozo)	1	14.38±1.90^{b}	19.44±2.72^{a}	11.50±1.19^{Bc}	17.30±3.14^{aB}	15.65±1.10^{B}
	2	19.94±2.32^{c}	15.52±2.86^{d}	21.25±2.56^{Ab}	27.52±4.17$^{A\,a}$	21.06±1.12^{A}
Mean		17.16±1.55^{b}	17.48±1.97^{b}	16.37±1.61^{b}	22.41±2.71^{a}	18.35
AST ($U/10^9$spermatozo)	1	36.83±5.34^{b}	34.11±3.45^{b}	27.11±3.9^{Bc}	48.64±6.57^{a}	36.67±1.16^{B}
	2	47.03±6.54^{a}	30.22±2.91^{b}	47.97±5.82^{Aa}	45.97±6.99^{a}	42.80±1.14^{A}
Mean		41.93±4.24^{a}	32.17±2.25^{c}	37.54±3.88^{b}	47.31±4.73^{a}	39.74
ALP ($U/10^9$spermatozo)	1	156.04±34.36^{c}	204.31±51.27^{b}	365.84±65.69^{a}	238.34±48.65^{b}	241.13±21.15
	2	201.28±45.4^{b}	265.68±57.88^{a}	297.73±44.1^{a}	270.9±47.46^{a}	258.80±28.16
Mean		178.66±28.33^{c}	234.99±38.46^{a}	331.79±39.43^{a}	254.62±33.61^{b}	249.97
ACP total ($U/10^9$spermatozo)	1	60.74±2.00	64.04±2.67	66.59±1.17	60.58±2.32	62.99±2.10
	2	60.16±2.03	60.15±2..28	60.03±2.89	60.71±1.00	60.26±1.84
Mean		60.45±5.07	62.09±5.43	63.31±5.56	60.65±4.93	61.62
ACP prostatic ($U/10^9$ spermatozoa)	1	3.42±0.16^{Bc}	5.64±0.85^{b}	9.09±0.80^{a}	4.53±0.65$^{B\,b}$	5.67±0.83^{B}
	2	16.19±0.84^{Ab}	13.17±0.94^{c}	24.12±0.26^{a}	14.37±0.64$^{A\,c}$	16.96±0.43^{A}
Mean		9.81±2.71^{b}	9.41±3.03^{b}	16.6±1.67^{a}	9.45±1.55^{b}	11.32

Control: untreated bulls, IVM: Ivermectin, LEV: Levamisole and ABZ: Albendazole.

a, b and c: Means in the same row with different superscripts differ significantly ($P<0.05$).

A, B: Means in the same column with different superscripts differ significantly

Data were represented by means ± SE.

Table(3): Effects of the different anthelmintics (ivermectin, levamisole and albendazole) administration of the first and second doses on chemical semen characteristics of Friesian bulls.

Item	No. of dose	treatment				Overall mean
		Control	IVM	LEV	ABZ	
Total proteins (g/dl)	1	5.48±0.62^{c}	7.47±1.02^{a}	5.52±0.7^{b}	3.68±0.51^{Bd}	5.54±0.53
	2	4.96±0.55^{c}	6.14±0.57^{a}	6.19±0.56^{a}	5.69±0.48^{Ab}	5.74±0.48
Mean		5.22±0.41^{bc}	6.8±0.59^{a}	5.85±0.44^{bc}	4.69±0.38^{c}	5.64
Albumin (g/dl)	1	1.97±0.29	2.44±0.29^{A}	2.21±0.48	1.72±0.31	2.08±0.28^{A}
	2	1.53±0.19	1.35±0.16^{B}	1.71±0.24	1.18±0.24	144±0.32^{B}
Mean		1.75±0.17	1.89±0.19	1.96±0.27	1.45±0.20	1.76
Globulin (g/dl)	1	3.51±0.33^{b}	5.03±0.73^{a}	3.31±0.22^{b}	1.96±0.20^{Bc}	3.45±0.20^{B}
	2	3.43±0.36^{b}	4.79±0.41^{a}	4.48±0.32^{a}	4.51±0.24^{Aa}	4.30±0.22^{A}
Mean		3.25±0.24^{a}	4.91±0.4^{a}	3.89±0.17^{a}	3.24±0.18^{a}	3.87
Creatinine (mg/dl)	1	0.62±0.07^{a}	0.49±0.09^{a}	0.57±0.03^{a}	0.25±0.05^{Bb}	0.48±0.08
	2	0.82±0.14	0.78±0.14	0.77±0.18	0.86±0.19^{A}	0.81±0.03
Mean		0.72±0.11^{a}	0.63±0.08^{a}	0.67±0.11^{a}	0.55±0.11^{b}	0.64
Urea (mg/dl)	1	78.50±8.40$^{a\,b}$	82.29±10.62^{a}	74.38±8.51^{Bb}	79.61±8.21^{aBb}	78.69±1.30^{B}
	2	93.99±9.48^{c}	110.32±12.31^{b}	122.58±11.15^{Aa}	112.21±10.37^{Ab}	109.77±2.13^{A}
Mean		86.24±6.38^{b}	96.30±8.35^{a}	98.48±8.02^{a}	95.91±7.08^{a}	94.23
Calcium (mg/dl)	1	26.46±2.08^{a}	26.87±1.24$^{A\,a}$	22.76±2.11^{ab}	20.81±1.78^{b}	24.22±1.10
	2	24.51±2.58	21.63±2.31^{B}	24.17±1.99	21.3±1.64	22.90±1.02
Mean		25.49±1.64^{a}	24.25±1.37^{a}	23.47±1.43^{ab}	21.05±1.20^{b}	23.56
Sodium (mmol/L)	1	101.67±6.43^{b}	93.2±11.23^{c}	84.99±18.48^{d}	112.44±6.71$^{a\,B}$	98.07±2.50^{A}
	2	108.92±6.81^{a}	76.49±6.17^{b}	104.27±7.24^{a}	81.89±12.85^{Ab}	92.89±2.81^{B}
Mean		105.29±4.63^{a}	84.84±6.54^{c}	94.63±9.90^{b}	97.16±7.9^{b}	95.48
Zinc (µg/dl)	1	243.00±6.00^{d}	389.00±5.00^{Ab}	341.00±5.00^{Ac}	607.00±6.00^{Aa}	395.17±6.80^{A}
	2	149.00±5.00^{b}	132.00±2.00^{Bc}	156.00±3.00^{Bb}	237.00±5.00$^{a\,B}$	168.70±5.11^{B}
Mean		196.00±6.50^{c}	260.00±3.50^{d}	249.00±4.00^{b}	422.00±5.50^{a}	281.93

Control: untreated bulls, IVM: Ivermectin, LEV: Levamisole and ABZ: Albendazole.

a, b and c: Means in the same row with different superscripts differ significantly ($P<0.05$).

A, B: Means in the same column with different superscripts differ significantly ($P<0.05$)

Data were represented by means ± SE

الملخص العربى

تأثير مضادات الطفيليات على الصفات البيوكيميائية للسائل المنوى المجمد للعجول الفريزيان

غاده عبد الحميد طبل* ، نبيله ابراهيم الدسوقى* ، محمد عبد الحليم تاج الدين** ، علاء السيد زيدان** ، رباب عبد المجيد كامل***

* قسم علم الحيوان- كلية العلوم- جامعة طنطا - مصر
** معهد بحوث الإنتاج الحيوانى- الدقى – الجيزة – مصر
*** كلية العلوم والآداب بالعارضة - قسم الفيزياء- المملكة العربية السعودية

تهدف الدراسه إلى المقارنه بين ثلاثة انواع من مضادات الطفيليات مختلفه فى طريقة عملها (افرمكتين- ليفاميزول- البندازول) من حيث تأثيرها على جودة السائل المنوى للعجول الفريزيان. استخدم فى هذه الدراسه عدد ١٦ عجل فريزيان تم تقسيمهم إلى اربعة مجاميع تتكون كلا منها من اربعة عجول. المجموعه الأولى مجموعه ضابطه، المجموعه الثانيه تم معالجتها بإفرمكتين (٢٠٠ ميكروجرام/كجم و حقت تحت الجلد)، المجموعه الثالثه تم معالجتها بليفاميزول (٧,٥مجم/كجم وتم حقنها تحت الجلد) والمجموعه الرابعه تم معالجتها بالبندازول (١٠مجم/كجم ، تجريع) كجرعه أولى ، حيث تم تجميع العينات على مدى ثمانية اسابيع ، ثم تم إعطاء الجرعه الثانيه من نفس العقاقيربنفس التركيزات السابقه وتم تجميع العينات على مدى ثمانية اسابيع أخرى. أظهرت الدراسه ان المعامله بالإفرمكتين احدثت تحسناً ملحوظاً فى الخواص الطبيعيه للسائل المنوى من حيث حجم القذفه وتركيزها والعدد الكلى للحيوانات المنويه فى كل قذفه ، كما احدثت تحسناً ملحوظاً فى الصفات الكيميائيه والتركيب الكيميائى لبلازما السائل المنوى .حيث أن الجرعه الأولى احدثت زياده ملحوظه فى انزيم الالنين امينوترانسفيراز والفوسفاتيز القاعدى والحامضى الناتج من غدة البروستاتا، كما زاد محتوى بلازما السائل المنوى من البروتين الكلى والجلوبيولين واليوريا وعنصر الزنك. كما ان المعامله بالجرعه الثانيه من افرمكتين احدثت نقص ملحوظ فى الإنزيمات الناقله لمجموعة الامين . المعامله بالليفاميزول ايضاً اوضحت زياده ملحوظه فى حجم قذفة السائل المنوى والعدد الكلى للحيوانات المنويه فى كل قذفه ، كما اظهرت نقصاً ملحوظاً فى نشاط الانزيمات الناقله لمجموعة الامين وزياده فى انزيمات الفوسفاتيز القاعدى والحامضى المفرز من غدة البروستاتا، ايضاً ظهرت زياده ملحوظه فى محتوى البلازما من البروتين الكلى ،الجلوبيولين، اليوريا والزنك. المعامله بالألبندازول سواءً بالجرعه الاولى او الثانيه احدثت نقصاً ملحوظاً فى الخواص الطبيعيه والكيميائيه للسائل المنوى ممثلة فى حجم القذفه وحيوية الحيوانات المنويه ، كما زاد نشاط الإنزيمات الناقله لمجموعة الامين زياده ملحوظه جداً ، وظهر نقص ملحوظ لمحتوى بلازما السائل المنوى من البروتين الكلى والجلوبيولين والكرياتينين والكالسيوم. كما حدثت زياده ملحوظه جداً لعنصر الزنك.

وبناءً عليه ومن الناحيه التطبيقيه يمكن أن نوصى بحقن طلائق التلقيح الاصطناعى بإفرمكتين (٢٠٠ميكروجرام/كجم) عند إجراء برامج التلقيح الاصطناعى لرفع نسبة الإخصاب. كما ان المعامله بمضادات الطفيليات (ليفاميزول) يجب ان تستخدم بحذر شديد فى موسم التناسل. يجب تجنب المعامله بالبندازول لما له من اثر سيء على جودة السائل المنوى ونقص فى حيوية الحيوانات المنويه.