REPRODUCTIVE PERFORMANCE OF FRIESIAN BULLS ADMINISTRATED WITH SELENOMETHIONINE, ZINCMETHIONINE OR THEIR COMBINATION

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SUMMARY

This investigation was to estimate the impact of daily oral treatments with some organic antioxidants as selenomethionine (Se-M) and zincmethionine (Zn-M), separately or together (Se-M plus Zn-M) to Fresian bulls through 150 days upon some blood components, libido, characteristics of fresh semen and frozen-semen thawed at various thawing regimes, as well as, fertilizing ability of bulls spermatozoa. Sixteen healthy Friesian bulls were separated into 4 groups. Bulls in the first, second, third and fourth groups were fed a basal diet, and given oral dose of 0, 0.3 mg Se-M, 4mg Zn-M, and 0.15mg Se-M plus 2mg Zn-M/kg of DM/bull/daily, respectively, for 150 days. The results elucidated that bulls were given Se-M plus Zn-M had optimal ($P<0.01$) values of hematological aspects, differential of leukocytic count, blood plasma testosterone, seminal plasma initial fructose, libido, as well as, physical aspects of fresh semen and frozen-semen thawed at various thawing rates comparing with the control or other treated bulls. Frozen-semen thawed at 55ºC for 15 seconds (rapid thawing rate) had best ($P<0.01$) quality, freezability and some biochemical components of seminal plasma and fertilizing ability of spermatozoa. In conclusion, daily oral dose of 0.15mg Se-M plus 2mg Zn-M/Kg DM/bull, as organic antioxidants through 150 days, could be suitable treatment for Friesian bulls, to achieving optimal quality of fresh semen production and freezability of semen, as well as, fertility of bulls spermatozoa, especially when frozen-semen thawed at 55º C for 15 seconds.

Keywords: *Friesian bulls, Semen cryopreservation, Thawing, Selenomethionine, Zincmethionine.*

INTRODUCTION

Many parts of the world are naturally deficient of micronutrients in soil and crop plants, particularly of zinc and selenium (Rashid and Ryan, 2008). Marginal deficiency of zinc of animals has been assessed in various regions in the tropical countries including various geological parts or most growing plants in Egypt (El-Fouly *et al.*, 1984), hence requires addition to the rations of livestock especially in organic form (Khalifa *et al.*, 2011). In Egypt, level of selenium in deferent feedstuffs may be lesser than the adequate concentrations set at 0.30 pp. (NRC, 2001) requirements. In addition, ruminants can't synthesis selenium or zinc in their bodies, thus an exogenous regular supply is needful for cover the physiological allowances and to sustain high productive performance (Ullrey, 1980).

Zinc is fundamental micronutrients present of small quantity in various cells and tissue of organisms (Kala *et al.*, 2016 and Zeweil *et al.* 2017) and plays a vital role of many biological functions, including DNA, RNA, protein synthesis and immune functions (Solomons, 1998), male fertility (Gabr, 2000) and metabolic activities (El-Hawary *et al.*, 2017). The importance of zinc for animal appears from the fact that zinc acts as an essential component and/or a cofactor of more than 200 metal enzymes and hormones (Smith and Akinbamizo, 2000). The high level of zinc is present in the male reproductive organs especially in prostate gland, epididymis, and testicles and in the spermatozoa itself, indicating to its essential role in protective the male reproductive tract and spermatozoa against harmful influence of reactive oxygen species (Gabr, 2000).

Zinc-methionine complex is absorbed in intestinal lumen and transported intact to mucosal cells, raising tissue supply with zinc and thereby enhancing animal performance (Hempe and Cousins, 1989).

Spermatogenesis needs some essential amino acids, particularly methionine, cysteine and/or arginine (Young *et al.*, 2008) and micronutrients, especially selenium and zinc (Cheah and Yang, 2011). Zinc addition through mineral amino acid chelates like organic forms which effectively had more bioavailability (Mandal *et al.*, 2008) and higher tissue concentrations (Cao *et al.*,2000), relative to inorganic forms. Bioavailability of zinc methionine to be 159% of the bioavailability of zinc sulphate in buffalo calves (Hassan *et al.*, 2016). Damascus goats bucks that were oral fed zincmethionine had significant ($P<0.01$) higher semen quality and blood testosterone level as well as better health status than the control bucks under hot summer season in Egypt (Abd-Allah *et al.*, 2021).

The important of selenium appears from the fact that selenium is found of all cells of animals and humans and it is a fundamental part of selenoprotein as (selenoprotein W or P and thiordoxine reductase, glutathione peroxidase and iodothyronine deiodinase), which plays a vital structural and/or enzymatic functions (Kassab and Mohammed, 2014).

Selenium has been exerted an immune modulatory impacts on the immune response (El-Hawary *et al.*, 2018). Also, selenium has free radicals scavenging activities both in vitro (Hussein, 2018) and in vivo (Abd El-Latif, 2001). Addition of selenium to the diet of sheep particularly in the organic form as selenized yeast led to enhancement of blood testosterone level and reproductive performance (Abd El-Hafez *et al.*, 2016). Testosterone level and/or function are significant ($P<0.01$) affected by selenium deficiency, which indicating to its important for biosynthesis of testosterone (Ghorbani *et al.*, 2018). High level of selenium is found of male reproductive system especially of the epididymis, suggesting its fundamental role in energy metabolis and/or maturation of spermatozoa (El-Seify, 2004). Selenium administration has been presented to improve semen quality through enhancing antioxidative defense mechanism of seminal plasma in buffalo bulls (Abd El-Latif, 2001) and in bucks (Shi *et al.*, 2010). Deficiency of selenium decreased the number and/or differentiation of germ cells which leading to reduce spermatogenesis (Sanchez-Gutierrez *et al.*, 2008). Also, there are relationship among selenium deficiency and damage of tail and head of spermatozoa and depressed of sperm motility (Safarinejad and Safarinejad, 2009).

Organic sources of selenium had more bioavailability (Antunovic *et al.*, 2014) lowest harmful and residual impacts in comparing with inorganic sources (Cao *et al.*, 2014). Supplementation of selenium or zinc in the ration of goat particularly in organic form, which led to improve antioxidant statues, testosterone and T_3 hormones of blood and/or plasma of goat (Kumar *et al.*, 2013). Addition of zincmethionine or selenomehionine to tris-yolk fructose extender throughout cryopreservation of bulls semen recorded the optimal post-thawing semen quality of bulls and conception rates for inseminated cows by using this semen (El-Hawary *et al.*, 2017).

Therefore, the specific purpose of this investigation was to clarify the influence of daily oral treatment of selenomethionine (Se-M), zincmethionine (Zn-M), or their combination (Se-M plus Zn-M), as organic antioxidants to Friesian bulls through 150 days upon blood components, libido, immune and health-status, endogenous antioxidants capacity, semen-production and quality of frozen-semen thawed at various thawing rates, as well as, fertilizing capacity of bulls spermatozoa.

MATERIALS AND METHODS

The present study was carried out at El-Gemmizah Experimental Station, El-Gharbiya governorate, belonging to the Animal Production Research Institute, Agricultural Research Center, Egypt.

Bulls and experimental design:

Sixteen healthy Friesian bulls with an average age of 3.5 – 4 years old and live body weight of 510 ± 40 kg were used in this investigation. They were randomly distributed into 4 similar groups (4 each) according to live body weight and age at the starting of the experiment. Bulls of the first group were fed on a basal ration without any addition and served as control group. While each bull of the second, third and fourth groups was fed on a basal ration and oral dose of 0.3mg selenomethionine (Se-M), 4 mg zinc-methionine (Zn-M), and 0.15mg Se-M plus 2mg Zn-M/ kg of dry matter (DM) / daily, respectively for 150 days, 60 days pre-semen collection and other 90 days as a main semen collection. All experimental bulls were kept separately under the same management and hygienic conditions. They were fed separately on a ration, which consisted of roughage as rice straw (RS) and clover hay (CH) and concentrate feed mixture (CFM), twice daily according to (NRC, 2001) recommendations, while

drinking water was offered all day time. The proximate chemical analysis of various feed stuffs (Table 1) was carried out according to A.O.A.C. (2016).

Table (1): Chemical analysis and feeding values of feed ingredients used in the basal diet.

Item	Ingredients of basal diet		
	Concentrate feed mixture	Clover hay	Rice straw
Chemical analysis (% on DM basis):			
Dry matter (DM)	90.50	90.31	91.50
Organic matter (OM)	90.56	88.95	83.94
Crude protein (CP)	15.98	16.31	3.48
Ether extract (EE)	3.11	2.51	1.43
Crude fiber (CF)	14.12	30.10	34.50
Nitrogen free extract (NFE)	57.35	40.03	44.53
Ash	9.44	11.05	16.06
Feeding values (% of DM):*			
Total digestible nutrients (TDN%)	62.07	63.33	54.95
Digestible crude protein (DCP%)	11.78	12.10	-0.21
Digestible energy (DE, Mcal/kg DM)	2.74	2.79	2.42
Metabloizable energy (ME, Mcal/kg DM)	2.32	2.37	1.99
Net energy (NE, Mcal/Kg DM)	1.40	1.43	1.23

DCP = 0.9596 CP- 3.55; TDN= 129.39 – 0.9419 (CF+NFE); DE+0.04409 (TDN%);ME= 1.01(DE)-0.45; NE= 0.0245(TDN%)-0.12, according to (NRC, 2001)

Blood sampling and analysis:

Blood samples were taken at the last week of the main semen collection period from each bull via the jugular vein in two heparinized centrifuge glass tubes. The first tube of each blood sample used for assessment of hematological measurements as total count of erythrocytes (RBC's), and leukocytes (WBC's), hemoglobin level (Hb), and haematocrite value (%), according to (Helper, 1966), also, differential leukocytes percentages were estimation according to (Lucky, 1977). The second tube of each blood sample was directly centrifuged at 3000 rpm for 15 minutes to separate blood plasma, which was kept at -20ºC in deep freezer until determination of testosterone level (Ekins, 1984), by radio-immunoassay (RIA) technique.

Collection, freezing and assessment of semen:

After 60 days as a preliminary period, semen ejaculates were collected once a week for 12 weeks as a main semen collection period from each bull of the experimental bulls by artificial vagina at about 6 – 7 a.m. prior feeding using teaser bull. On each collection day, libido (reaction time, seconds) was counted for each bull according to (Chenoweth, 1981), and volume of semen ejaculate was computed directly after ejaculation in a transparent graduated glass collecting tube. Collecting tubes containing semen ejaculates were kept separately in a water bath at 37ºC and immediately transferred into the laboratory for assessment of fresh semen quality.

On each day of collection semen, semen ejaculates that were taken from all bulls in the same treatment were pooled and just diluted with Tris-egg yolk fructose (TEYF) at a rate of 1 semen : 8 extender (v/v) at 37ºC. TEYF extender was consisted of (3.028g tris amino methane; 1.25g fructose; 1.675g citric acid; 10% egg yolk; 7% glycerol; 500µg streptomycin and 500 IU penicillin and completed with distilled water up to 100 ml, Salisbury *et al.,* 1978). Diluted semen samples were kept at 5ºC for 6 hours as an equilibration time and packaged in Franch straws (0.50 ml capacity), then the filled straws were sealed by heat and freezed in liquid nitrogen container for storage at -196ºC (Salisbury *et al.,* 1978) for 4 weeks, thereafter frozen semen in straws were thawed in water bath at various thawing regimes (slow thawing: 15ºC for 60 sec., moderate thawing: 35ºC for 30 sec. and rapid thawing: 55ºC for 15 sec.) for post thawing semen evaluation.

Percentages of sperm motility, normality or livability were assessed (Salibury *et al.*, 1978), as well as, percentages of sperm with acrosomal damage were evaluated by using Giemza stain (Watson, 1975), in both fresh semen and post thawing semen at various thawing regimes. Sperm cell concentration was

computed using heamocytometer slide (Khan, 1994), and total sperm output was also calculated for each semen ejaculate of each bull.

Initial seminal fructose was assessed immediately post collection of semen ejaculates (Barakat and El-Sawaf, 1964). Frozen thawed semen samples at various thawing regimes were centrifuged at 3000 rpm for 15 minutes, then clear seminal plasma was aspirated and stored at -20ºC, until time of assay. Estimation of total proteins level was carried out collorimetrically using Biuret method (Weichselbaum, 1946), and albumin was measured collourimetrically by bromocresol green method (Doumas *et al.*, 1971), while globulin value was computed by subtracting albumin from total proteins contents. The activity of hyaluroindase enzyme was measured after suitable dilution with distilled water according to (Foulkes and Watson, 1975). Malondialdehyde was measured according to (Richard *et al.*, 1992), while total antioxidant capacity (TAC) was assayed (Koracevic *et al.*, 2001).

Fertility rate:

Sixty-four Friesian dairy cows in normal estrous and heat were randomly distributed into 4 groups (16/each). Cows in all groups were artificially inseminated (AI) with Frozen-thawed semen at 55ºC for 15 seconds (the best thawing regimes) of control bulls and administrated with 0.3 mg Se-M, 4 mg Zn-M, and 0.15 Se-M plus 2 mg Zn-M/kg DM/daily, respectively. For each cow, two inseminations were performed, one in the morning and another in the evening, with straw (0.25 ml) of frozen-thawed semen, by recto-vaginal insemination technique (Salisbury *et al.*, 1978). Fertility rate was performed on the basis of pregnancy diagnosis by rectal examination following 60 days from the day of insemination.

Statistical analysis:

The obtained data were subjected to statistical analysis by using analysis of variance procedure (SPSS, 2013). The significant differences among means were assesses Duncan's multiple range test (Duncan, 1955). The percentage values were transferred into arc-sing prior being analysis and computed as means. The conception rates results were also analysed using chi-square test.

RESULTS AND DISCUSSION

Hematological aspects:

In Table (2), values of hemoglobin, RBC's or WBC's and hematocrit % for bulls were affected ($P<0.01$) by various treatments. Bulls that were fed Se-M plus Zn-M together recorded the best ($P<0.01$) positive influence on all hematological aspects of bulls, exhibiting synergic influence for Se-M plus Zn-M administration on blood hematological aspects of bulls. These findings are similar to that obtained by (Hassan *et al.*, 2016; Zeweil *et al.*, 2017 and El-Nagar *et al.*,2021). In general consistent with the current research, natural organic antioxidants treatments have powerful influence on hematological characteristics of various species, including the influence of selenomethionine on buffalo-cows (El-Hawary *et al.*, 2018) and zincmethionine on Damascus goat bucks (Abd-Allah *et al.*,2021). El-Hawary *et al.* (2018) who stated that feeding buffalo cows a ration added with selenomethionine throughout the transition period enhanced ($P<0.05$) values of WBC's, RBC's and a haematocrite in comparing with the control cows.

With relationships to differential of white cell count of bulls, treatment of Se-M plus Ze-M to bulls evoked a highly significant decline ($P<0.01$) in neutrophils%, and a highly significant enhance ($P<0.01$) in lymphocytes% when compared with that of control or other treatments. On the other hand, there was no significant variation in monocytes%, eosinophils%, and basophilis% between supplemented groups. The obtained results go in hand with the findings obtained by (Khalifa *et al.*, 2011; Kala *et al.*, 2016 and Abd Allah, 2022). Also, selenium exerts an immunomodulatory influences on the immune response (Cortinhas *et al.*, 2012). Furthermore, these results can be explained by observation that zinc and/or selenium, especially organic forms are essential trace element, which share in common biological role as antioxidants which preventing all cell membranes in the body including cell membranes of lymphoid cells from oxidative damage by free radical (El-Tohamy *et al.*, 2012 and Cao *et al.*, 2014). Another plausible explanation recorded by Mamdouh (2000) stated that there was as increased migration of lymphocytes from the thymus gland under the influence of selenium plus vitamin E treatment. Fukada *et al.* (2011) and Zewil *et al.* (2017) demonstrated that insufficient amount of zinc for animal impaired functions of natural killer cells, T or B cells, macrophages and neutrophils (phagocytosis). Finally, the observed enhancement in hematological aspects of bulls by administration of selenomethionine and zincmethionine separately or together in our investigation mainly related to the positive influence of

these administration as organic antioxidants by protecting against reactive oxygen species (ROS) generation of hematopoietic cells.

Table (2): Influence of various treatments on hematological aspects and differential of white cell count of bulls.

Statements	Treatments			
	Control	Se-M	Zn-M	Se-M + Zn-M
Hematological measurements:				
Haemoglobin (mg/dl)	6.98 ± 0.35^{c}	9.25 ± 0.25^{b}	9.13 ± 0.23^{b}	12.10 ± 0.20^{a}
RBC's (x 10^6/mm^3)	6.85 ± 0.25^{c}	8.70 ± 0.15^{b}	8.56 ± 0.13^{b}	9.95 ± 0.11^{a}
WBC's (x 10^6/mm^3)	6.89 ± 0.21^{c}	8.46 ± 0.13^{b}	8.70 ± 0.14^{b}	10.20 ± 0.12^{a}
Haematocrite (%)	30.44 ± 0.51^{c}	37.13 ± 0.25^{b}	35.55 ± 0.20^{b}	45.11 ± 0.15^{a}
Differential of White blood cells:				
Neutrophils (%)	45.60 ± 0.79^{a}	28.19 ± 1.13^{b}	29.11 ± 1.31^{b}	23.17 ± 1.36^{c}
Lymphocytes (%)	40.55 ± 0.84^{c}	57.67 ± 0.60^{b}	58.40 ± 0.51^{b}	64.15 ± 0.45^{a}
Monocytes (%)	6.95 ± 0.25	6.94 ± 0.17	5.89 ± 0.20	5.85 ± 0.09
Eosinophils (%)	6.45 ± 0.30	6.77 ± 0.19	6.13 ± 0.21	6.50 ± 0.18
Basophils (%)	0.45 ± 0.20	0.43 ± 0.17	0.47 ± 0.18	0.33 ± 0.10

a,b and c: Group differences within each row at (P<0.01)

Fresh semen measurements:

Results in Table (3) declared that oral administration of Se-M and/or Zn-M recorded a high significant improved (P<0.01) of all fresh semen measurements of bulls when comparing with the control-untreated bulls. The optimal (P<0.01) improvements were assessed with bulls that were oral fed Se-M plus Zn-M together compared with untreated or other administrated bulls. These findings are consistent with Abdel-Khalek *et al.* (2010) and El-Hawary (2010) in buffalo bulls. Also, Abd-Allah *et al.* (2021) found that treatment with zincmethionine enhanced (P<0.01) quality of fresh semen of Damascus goat bucks throughout summer season in Egypt.

The highly enhancement for aspects of fresh semen for treated bulls were mainly response to anti-oxidants properties of Se-M and Zn-M as organic antioxidants, and synergistic impacts of their combination on antioxidant defense mechanism by preventing of all body cells from harmful influence of free radicals, which are producing by cellular metabolism for all body cells under normal or stress conditions. In this respect, El-Tohamy *et al.* (2012); Ghorbani *et al.* (2018) and Abd-Allah (2022) in bucks, who stated that zincmethionine supplementation improved the activities of glutathione peroxidase (GSH) and superoxide dismutase (SOD) enzymes in blood and seminal plasma of supplemented animals under heat stress, which are the main defense mechanism against harmful influence for free radicals.

Dietary addition with methionine had powerful influence on quality of semen through increasing the activities of glutathione peroxidase enzyme in intracellular of spermatozoa of buffalo bulls (Singh *et al.*, 2000). Furthermore, spermatogenesis needs to some minerals, especially selenium and/or zinc (Cheah and yang, 2011).

Table (3): Influence of various treatments on physical aspects of fresh semen of bulls.

Statements	Treatments			
	Control	Se-M	Zn-M	Se-M + Zn-M
Ejaculated volume (ml)	2.25 ± 0.25^{c}	3.15 ± 0.20^{b}	3.48 ± 0.16^{b}	4.31 ± 0.15^{a}
Sperm motility (%)	71.76 ± 2.23^{c}	80.66 ± 1.55^{b}	85.50 ± 1.99^{b}	91.11 ± 0.81^{a}
Sperm livability (%)	72.11 ± 2.40^{c}	81.76 ± 1.44^{b}	83.50 ± 1.55^{b}	95.53 ± 1.88^{a}
Sperm normality (%)	80.29 ± 0.75^{c}	85.53 ± 1.15^{b}	88.40 ± 1.04^{b}	96.70 ± 1.30^{a}
Acrosomal damage (%)	20.11 ± 0.43^{a}	15.80 ± 0.50^{b}	14.81 ± 0.25^{b}	9.43 ± 0.15^{c}
Sperm concentration (x10^9/ml)	0.99 ± 0.05^{c}	1.23 ± 0.03^{b}	1.30 ± 0.04^{b}	1.60 ± 0.06^{a}
Total sperm output (x10^9/ejac.)	2.23 ± 0.23^{c}	3.87 ± 0.30^{b}	4.52 ± 0.34^{b}	6.90 ± 0.21^{a}

a,b and c: Group differences within each row at (P<0.01)

In addition, these finding may be response to raising of testosterone concentration in blood of bulls that were oral fed Se-M and/or Zn-M (Table 4), which resulted in producing excellent semen quality, being the powerful efficient with bulls that were oral fed Se-M plus Ze-M together. These results are

consistent with El-Siefy (2004); Kumar *et al.* (2013) and Abd-Allah *et al.* (2021). El-Gohary *et al.* (2008) stated that concentration of testosterone in blood is very needful for male reproductive, it is relationships to development of reproductive organs, the maintenance of sexual activities, spermatogenesis and semen quality. Abd-Allah *et al.* (2021) stated that addition of zincmethionine to Damascus goat bucks improved blood testosterone level and their semen quality in comparing with the control.

Blood testosterone, seminal plasma fructose and libido of bulls:

Results tabulated in Table (4) showed that a highly significant effect (P<0.01) on blood plasma testosterone level and initial fructose level in seminal plasma, as well as, sex drive expressed as reaction time per second due to treatments. Furthermore, superior beneficial influence was assessed for bulls that were supplemented with a combination of Se-M plus Zn-M under this experiment conditions. Although the variances influence within bulls that were received Se-M or Zn-M on all above parameters were insignificant, they were better for bulls that were given Zn-M alone. Such findings indicated that treatment of Se-M plus Zn-M together to bulls had a positive synergistic influence on blood plasma testosterone level and initial fructose in seminal plasma, as well as, libido of bulls. These findings are in accordance with El-Seify (2004); Kala *et al.* (2016) and Ghorbani *et al.* (2018). Also, El-Tohamy *et al.* (2012) and Abd-Allah *et al.* (2022) in bucks, who stated that addition of zincmethionine had achieved significant reduction (P<0.01) of reaction time and marked enhance (P<0.01) of blood testosterone level when compared with the control one under hot summer conditions in Egypt. Abd El-Hafez *et al.* (2016) in male lambs, who reported that testosterone levels in group treated with organic selenium as selenized yeast was significant increase comparing with that in the group treated with sodium selenite or untreated group. El-Gohary *et al.* (2008) and Kala *et al.* (2016) documented that blood testosterone level is very needful for male reproductive, it is relationships to development of reproductive organs, the maintenance of sexual activities, spermatogenesis and semen quality.

The detectable increased (P<0.01) of initial fructose for fresh semen in all administered bulls was mainly relationships to a higher activities of the accessory sexual glands in response to direct influence of treatments, especially with Se-M plus Zn-M combination, which recorded the highest (P<0.01) level of initial fructose in fresh semen. Our finding is consistant with Iman *et al.* (2009) in buffalo bulls and Ghorbani *et al.* (2018) in mature rams. Abdel-Khaled *et al.* (2010) who documented that there was a close relationship among citric acid and fructose levels in seminal plasma and male testosterone level and/or activity. El-Siefy (2004) and Abd Allah (2022) stated that fructose level in seminal plasma reflects quality of semen due testosterone activities of male.

Table (4): Influence of various treatments on blood plasma testosterone and seminal plasma fructose levels and libido of bulls.

Statements	Treatments			
	Control	Se-M	Zn-M	Se-M + Zn-M
Testosterone (ng/ml)	0.69 ± 0.20^{c}	1.07 ± 0.18^{b}	1.60 ± 0.12^{b}	2.13 ± 0.04^{a}
Fructose levels (mg/100ml)	319.20 ± 20.30^{c}	440.50 ± 15.30^{b}	485.11 ± 13.40^{b}	560.71 ± 15.18^{a}
Libido (seconds)	155.28 ± 0.67^{a}	64.95 ± 4.38^{b}	58.24 ± 3.41^{b}	30.27 ± 2.11^{c}

a,b and c: Group differences within each row at (P<0.01)

Freezability of bulls spermatozoa:

Results in Table (5) revealed that bulls that were treated with Se-M and/or Zn-M produced excellent semen quality containing spermatozoa characteristics by a highly significant (P<0.01) improved of post-thawing motility, livability, normality and acrosmal damage when compared with untreated bulls. Finally, bulls that were received Se-M plus Zn-M combination recorded the maximal benefits on quality and freezability of cryopreserved semen, which are in harmony with semen physical aspects of the same bulls in fresh semen. These findings are in agreement with those of (El-Sheltawi *et al.*, 1999; El-Siefy, 2004; Pankaj *et al.*, 2014 and Hussein, 2018). Buffalo bulls supplemented with zinc and selenium showed significant better of sperm motility, intact acrosome and livability of post-thawed semen with greatest conception rate of buffalo-cows, El-Hawary (2010).

With related to thawing regimes, the optimal percentages (P<0.01) of post-thawing motility, livability, normality and acrosomal damage of bulls spermatozoa were assessed with thawing rate of 55ºC for 15 seconds when compared with 35ºC for 30 seconds or 15ºC for 60 seconds. Curry and Watson (1994) indicated that when frozen-semen was thawed fastly, re-crystallization of water molecules may be

avoided. Zeidan *et al.* (2004) and El-Shennawy (2013) in Friesian bulls and Gabr (2009) and Kobaide (2016) in buffalo bulls spermatozoa, who emphasized rapid thawing of semen at 65ºC for 10 second or moderate thawing at 35ºC for 30 seconds was important for achieving optimal, freezability and fertility of bulls spermatozoa.

Table (5): Influence of various treatments and thawing rates on characteristics of bulls spermatozoa.

Statements	Treatments	Thawing rates (ºC/second)			Overall mean
		15 ºC/60sec.	35 ºC/30sec.	55 ºC/15sec.	
Sperm motility (%)	Control	25.10 ± 3.50	35.70 ± 3.30	43.50 ± 3.06	34.77 ± 2.90^{c}
	Se-M	36.91 ± 1.40	43.10 ± 2.10	47.63 ± 2.90	42.55 ± 1.80^{b}
	Zn-M	38.98 ± 1.30	46.54 ± 1.20	50.45 ± 1.70	45.32 ± 0.99^{b}
	Se-M+Zn-M	40.30 ± 1.40	49.36 ± 1.20	59.98 ± 0.90	49.88 ± 0.73^{a}
Overall mean		35.33 ± 2..70^{c}	43.67 ± 2.18^{b}	50.39 ± 0.88^{a}	
Sperm livability (%)	Control	30.20 ± 3.15	36.40 ± 3.90	40.33 ± 3.05	35.64 ± 2.90^{c}
	Se-M	45.31 ± 1.31	48.35 ± 1.10	50.41 ± 2.10	48.02 ± 1.03^{b}
	Zn-M	47.51 ± 0.90	48.73 ± 1.00	53.88 ± 0.85	50.04 ± 0.98^{b}
	Se-M+Zn-M	50.31 ± 0.71	58.88 ± 0.93	68.15 ± 0.54	59.11 ± 0.60^{a}
Overall mean		43.33 ± 2.01^{c}	48.09 ± 1.05^{b}	53.19 ± 0.88^{a}	
Sperm normality (%)	Control	51.10 ± 2.40	56.80 ± 2.10	58.15 ± 0.95	55.35 ± 2.15^{c}
	Se-M	59.51 ± 1.15	62.54 ± 0.98	69.71 ± 0.77	63.92 ± 0.89^{b}
	Zn-M	60.40 ± 1.01	64.80 ± 0.90	73.98 ± 0.60	66.40 ± 0.73^{b}
	Se-M+Zn-M	63.50 ± 0.31	73.11 ± 0.25	85.10 ± 0.20	73.90 ± 0.41^{a}
Overall mean		58.63 ± 1.80^{c}	64.31 ± 1.04^{b}	71.74 ± 0.50^{a}	
Acrosomal damage (%)	Control	49.00 ± 1.70	40.17 ± 1.60	39.01 ± 1.40	42.72 ± 1.31^{a}
	Se-M	30.40 ± 0.80	29.80 ± 0.68	26.15 ± 0.50	28.78 ± 0.70^{b}
	Zn-M	33.50 ± 0.90	26.20 ± 0.77	21.53 ± 0.43	27.07 ± 0.63^{b}
	Se-M+Zn-M	25.00 ± 0.51	20.18 ± 0.41	15.40 ± 0.31	20.19 ± 0.32^{c}
Overall mean		34.47 ± 0.99^{a}	29.08 ± 0.81^{b}	25.52 ± 0.23^{c}	

a,b and c: Group differences within each row or column at (P<0.01)

Seminal plasma proteins:

Table (6) illustrated that values of seminal plasma total proteins and albumin were affected (P<0.01) by various treatments to bulls. Finally, bulls that were oral fed Se-M plus Zn-M together had significant optimal influence on seminal plasma total proteins and albumin. It is worth noting that, various administrations didn't significant influences on seminal plasma globulin concentration.

Dhami *et al.* (1994) and Kulkarni *et al.* (1996) who confirmed that total proteins in seminal plasma are mainly consisted of albumin in a high degree and globulin to a lesser degree as well as small quantities of peptides, amino acids and nonprotein nitrogen, thus compounds make up the amphoteric property of seminal plasma proteins and, so, low protein content in seminal plasma decrease its buffering capacity and in turn quality of semen. Taha *et al.* (2000) and Osama and El-Sahn (2006) stated that the positive influence of seminal plasma proteins on enhancing semen ejaculate volume, sperm concentration and motility reside in their content of albumin or specific factors in seminal plasma.

The current investigation demonstrated that there was beneficial relationship among increasing concentration of total proteins and albumin in seminal plasma and improving semen quality measurements in post-thawing semen for bulls that were received Se-M and/or Zn-M, especially with Se-M plus Zn-M combination, which recorded the optimal values. In accordance with Table *et al.* (2012) found that the marked improvement in post-thawing sperm motility and livability are associated with increased level of total proteins and albumin in seminal plasma. Also, ElKomy *et al.* (2008) who stated that improve of levels of total protein and/or albumin in seminal plasma were assessed in excellent fertile rabbits compared with low fertile rabbits, and this improve as associated with improve their seminal quality characteristics.

The significant enhancement of seminal plasma content of total proteins and albumin of bulls that were received various treatments, especially with Se-M plus Zn-M together may be reside in the enhance of methionine content in Se-M plus Zn-M, which needful for synthesis of protein. Our findings are supported by Singh *et al.* (2000); El-Siefy (2004); El-Shennawy (2013) and Ghorbani *et al.* (2018).

Table (6) showed that total proteins and albumin content in seminal of post-thawed semen of treated bulls was affected (P<0.01) by thawing regimes, being the optimal (P<0.01) when thawing was carried out at 55º C for 15 seconds. These findings are in accordance with Almquist and wiggin (1973); Gabr (2009) and Kobaide (2016).

Table (6): Influence of various treatments and thawing rates on total proteins and its fractions in seminal plasma of post-thawed semen of bulls.

Statements	Treatments	Thawing rates (ºC/second)			Overall mean
		15 ºC/60sec.	35 ºC/30sec.	55 ºC/15sec.	
Total proteins (g/dl)	Control	2.93 ± 0.80	2.98 ± 0.70	4.35 ± 0.41	3.42 ± 0.51^{c}
	Se-M	4.68 ± 0.37	4.88 ± 0.40	5.99 ± 0.32	5.18 ± 0.41^{b}
	Zn-M	5.53 ± 0.44	5.63 ± 0.50	6.41 ± 0.21	5.85 ± 0.38^{b}
	Se-M+Zn-M	6.44 ± 0.30	6.54 ± 0.33	7.47 ± 0.15	6.81 ± 0.29^{a}
Overall mean		4.89 ± 0.60 ^{c}	5.01 ± 0.71^{b}	6.05 ± 0.34^{a}	
Albumin (g/dl)	Control	1.50 ± 0.30	1.60 ± 0.20	3.20 ± 0.19	2.10 ± 0.41^{c}
	Se-M	2.95 ± 0.21	3.11 ± 0.24	3.80 ± 0.22	3.29 ± 0.20^{b}
	Zn-M	3.77 ± 0.20	3.90 ± 0.19	4.20 ± 0.17	3.95 ± 0.31^{b}
	Se-M+Zn-M	4.05 ± 0.18	4.21 ± 0.17	5.04 ± 0.15	4.43 ± 0.15^{a}
Overall mean		3.07 ± 0.20^{c}	3.21 ± 0.10^{b}	4.06 ± 0.13^{a}	
Globulin (g/dl)	Control	1.43 ± 0.29	1.38 ± 0.18	1.15 ± 0.17	1.32 ± 0.20
	Se-M	1.73 ± 0.18	1.77 ± 0.16	2.19 ± 0.15	1.89 ± 0.15
	Zn-M	1.76 ± 0.15	1.73 ± 0.14	2.21 ± 0.12	1.90 ± 0.11
	Se-M+Zn-M	2.39 ± 0.08	2.33 ± 0.09	2.43 ± 0.07	2.38 ± 0.04
Overall mean		1.82 ± 0.21	1.80 ± 0.13	1.99 ± 0.10	

a,b and c: Group differences within each row or column at (P<0.01)

Lipid peroxidation and antioxidative status:

It was noted that hyaluronidase activity and malondialdehyde (MDA) level reduced (P<0.01), while total antioxidant capacity (TAC) concentration enhanced (P<0.01) in seminal plasma of frozen-thawed semen of bulls by treatments, being the optimal (P<0.01) values with bulls that were oral fed Se-M plus Zn-Met together (Table 7). Similar findings were noted by (El-Hawary, 2010; Shi *et al.* 2010 and Ghorbani *et al.*, 2018). Also, Zeweil *et al.* (2017) and Abd Allah (2022) confirmed that orally zinc administration had significantly increased TAC concentration and significantly reduced MDA level which used as lipid peroxidation index in comparing with control. The marked reduction of hyaluronidase activity and MDA level in seminal plasma of post-thawed semen of bulls in different treatments, especially with Se-M plus Zn-M together mainly related to improve of TAC in seminal plasma, which may provide better protective to spermatozoa from harmful influence of reactive oxygen species due to lipid peroxidation. El-Sheltawi *et al.* (1999); Abd El-Latif (2001) and El-Hawary (2010) reported that in vivo administration of zinc plus vitamin E proved to protected the cell membrane of buffalo bull spermatozoa during freezing and thawing as indicated from the lowest amount of enzymes releasing from spermatozoa to extracellular medium. Also, El-Nagar *et al.* (2021) found that bulls that were oral received N-acetylcysteine plus L-carnitine, as organic antioxidant had reduced the rate of lipid peroxidation and improved the antioxidatives properties in seminal plasma of post-thawed semen of bulls.

Finally, treatment with combination of organic antioxidants as Se-M plus Zn-M had the highest protective against lipid peroxidation and/or oxidative stress injury to keep the structural integrity of frozen-thawed bulls spermatozoa membrane preventing release of intracellular enzymes into seminal plasma.

With related to thawing rates, frozen semen thawed at 55ºC for 15 seconds (fasting thawing rate) maintained significantly (P<0.01) lower level of MDA and leakage of hyaluronidase enzyme, and significantly (P<0.01) higher TAC level in seminal plasma than 35ºC for 30 second (moderate thawing rate) or 15ºC for 60 seconds (slow thawing rate). No significant differences were detected in the levels of hyaluronidase enzyme, MDA and TAC between the frozen-semen thawed at 35ºC for 30 seconds (moderate thawing rate) and 15ºC for 60 seconds (slow thawing rate).

An appropriate rate of thawing in term of temperature and time is needed to prevent re-crystalization in post-thawed spermatozoa. (Zeidan *et al.*, 2004 Gabr, 2009 and Kobaide, 2016) observed that fasting thawing rate avoids the possibility of re-crystalization of water molecules, which could be injurious to cell membrane of spermatozoa. The optimal thawing rate with antioxidant addition to bovine and buffalo semen was suggested to be 55ºC for 15 seconds (rapid thawing rate) was found to affect sperm quality (Almquist and wiggin 1973 and Gabr, 2009), respectively.

Table (7): Influence of various treatments and thawing rates on hyaluronidse enzyme, malondialdehyde and total antioxidants capacity in seminal plasma of post-thawed semen of bulls.

Statements	Treatments	Thawing rates (ºC/second)			Overall mean
		15 ºC/60sec.	35 ºC/30sec.	55 ºC/15sec.	
Hyaluronidse ($U/10^9$ spermatozoa)	Control	98.17 ± 13.11	95.94 ± 12.14	70.18 ± 10.40	88.10 ± 12.15[a]
	Se-M	81.33 ± 8.70	89.54 ± 5.73	61.83 ± 8.31	77.56 ± 7.35[b]
	Zn-M	79.89 ± 7.88	74.37 ± 4.55	58.39 ± 4.15	70.88 ± 6.99[b]
	Se-M+Zn-M	60.81 ± 5.17	58.93 ± 3.41	45.38 ± 3.16	55.04 ± 3.17[c]
Overall mean		80.05 ± 6.98[a]	79.69 ± 5.73[a]	58.95 ± 2.99[b]	
Malondi-aldehyde (n mol/ml)	Control	75.50 ± 2.11	70.11 ± 1.90	60.40 ± 1.60	68.67 ± 1.87[a]
	Se-M	65.30 ± 1.10	61.81 ± 1.40	53.18 ± 0.77	60.10 ± 1.11[b]
	Zn-M	60.11 ± 1.05	58.19 ± 1.18	45.37 ± 0.90	54.55 ± 1.32[b]
	Se-M+Zn-M	49.31 ± 0.9	45.89 ± 0.85	35.77 ± 0.80	43.65 ± 0.75[c]
Overall mean		62.56 ± 1.40[a]	59.00 ± 1.21[a]	48.68 ± 0.87[b]	
Total antioxidants capacity (mM/l)	Control	50.50 ± 1.15	55.13 ± 1.01	65.15 ± 0.91	56.93 ± 0.99[c]
	Se-M	65.31 ± 0.90	66.45 ± 0.85	99.50 ± 0.60	77.08 ± 0.55[b]
	Zn-M	70.92 ± 0.83	73.83 ± 0.71	105.11 ± 0.80	83.28 ± 0.60[b]
	Se-M+Zn-M	95.81 ± 0.60	98.91 ± 0.63	120.50 ± 0.81	105.07 ± 0.76[a]
Overall mean		70.64 ± 0.88[b]	73.57 ± 0.76[b]	97.56 ± 0.66[a]	

a,b and c: Group differences within each row or column at (P<0.01)

Fertility rates of cows:

As presented in Table (8), the current investigation illustrated that conception rates (%) of cows inseminated with frozen thawed semen at 55ºC for 15 seconds (the best thawing regimes) were affected (P<0.01) by Se-M or Zn-M singly or combination (Se-M+Zn-M) treatments to bulls. Furthermore, frozen thawed semen at 55ºC for 15 second of bulls that were given Se-M plus Zn-M together had optimal (P<0.01) influence on conception rates of inseminated cows when compared with those inseminated with frozen thawed semen at 55ºC/15 seconds of bulls that were given Se-M or Zn-M separately, and the control bulls. However, the conception rate (%) for cows in inseminated with thawed semen at 55ºC for 15 seconds of bulls that were oral fed with Se-M or Zn-M alone was insignificant, but it was higher in cows artificially inseminated with semen from bulls that were received Zn-M.

Table (8): Conception rates (%) for cows artificially inseminated with frozen-thawed semen at 55ºC for 15 seconds from treated bulls.

Statements	Treatments			
	Control	Se-M	Zn-M	Se-M + Zn-M
Number of inseminated cows	16	16	16	16
Number of conceived cows	8	10	11	14
Conception rate (%)	50.00[c]	62.50[b]	68.75[b]	87.50[a]

a,b and c: Group differences within each row at (P<0.01)

Finally, the highest (P<0.01) values of fertility rates (%) of cows artificially inseminated with frozen-thawed semen at 55ºC for 15 seconds of bulls that were orally supplemented with Se-M plus Zn-M combination were mainly related to optimal enhancements of general health statues and immunity response of bulls (Table 2), or raising of blood testosterone level of bulls (Table 4) in response to oral treatment of Se-M plus Zn-M together, which resulted in producing excellent semen having the best (P<0.01) values of physical semen aspects (Table 3), post-thawed quality (Table 5), as well as, the optimal seminal plasma constituents in post-thawed semen of bulls (Table 6 and 7), respectively. Similar

results were obtained by (Zeidan *et al.*, 2004; El-Hawary, 2010 and El-Shennawy, 2013). Zeidan *et al.* (2004) and Gabr (2009) reported that fast thawing rate for semen straws was vital for achieving maximal improvement of fertilizing efficiency of bull's spermatozoa. In this respect, the evident advantage of rapid thawing rate of frozen-semen in straws could be explained on the basis that each straw makes a certain special plastics coat around the spermatozoa which needs a high thawing temperature (Almquist and Wiggin, 1973 and El-Shennawy, 2013).

CONCLUSION

In conclusion, daily oral dose of 0.15mg Se-M plus 2mg Zn-M/Kg DM/bull, as organic antioxidants through 150 days, could be suitable treatment for Friesian bulls, to achieving optimal quality of fresh semen production and freezability of semen, as well as, fertility of bulls spermatozoa, especially when frozen-semen thawed at 55º C for 15 seconds.

REFERENCES

Abd-Allah M. S.; El-Kelawy, H.M.; Ibrahim, H.; El-Gaafary M.N. and Abou El-Ela, A. A. (2021). Effect of treatment with L-Carnitine, co-enzyme Q10 and zinc-methionine as stimulants on semen quality and some blood parameters of Damascus goat bucks during summer season of Egypt. J. Product. and Dev., 26(4): 671- 688.

Abd-Allah, M. S. (2022). Using some feed additives to improve productive and reproductive performance of Damascus goats under Egyptian environmental conditions. Ph.D, thesis, Faculty of Technology and Department, Zagazig University, Zagazig, Egypt.

Abd El-Latif, M.A.R. (2001). Effect of selenium and vitamin E on semen characteristics of buffalo bulls. M. Sc. Thesis, Fac., Agric., Minufiya Univ., Egypt.

Abd El-Hafez, G.A.; Solouma, G.M.A.; Kassab, A.Y. and Ali, A.S. (2016). Some reproductive performance of male lambs and feeding values of rations as affected by supplementation of different selenium sources. Egypt. J. Nutr. and feeds, 19(1): 103 – 114.

Abdel-Khalek, A.E.; Yousf, M.M. and El-Hawary, A.F. (2010). Sexual desire, testicular measurements and semen quality of buffalo bulls treated with combinations of trace elements and vitamin E. J. of Animal and Poultry Production, 1 (3): 97 – 107.

Almquist, J.O. and Wiggin, H.B. (1973). Effect of different combinations of freezing and thawing rates upon survival of bull spermatozoa in United States plastic straws. Artificial Insemination Digest, 21: 10.

Antunovic, Z. Novoselec, J.; Speranda, M.; Klapec, T.; Avar, S.; Mio, B. and Vukovi, R. (2014). Influence of dietary supplementation with selenium on blood metabolic profile and thyroid hormones activities in fattening lambs. Pakistan Veterinary Journal. 34(2): 224 -228.

A.O.A.C. (2016). *Association Of Official Analytical Chemists*. Official methods of analysis, 20 th Ed. USA, Washington, .USA.

Barakat, M.Z. and El-Sawaf, E. (1964). The determination of semen fructose. Micro-chemical J., 8: 123.

Cao, J.; Henry, P.R. Guo, R.; Holwerda, R.A.; Troth, J.P.; Littell, R.C.; Miles, R.D. and Ammerman, C.B. (2000). Chemical characteristics and relative bioavability of supplemental organic zinc sources for poultry and ruminants. . J. Anim. Sic., 78: 2039 – 2054.

Cao, J.; Guo, F.; Zhang, L.; Dong, B. and Gong, L. (2014). Effects of dietary selenomethionine supplementation on growth performance, antioxidant status, plasma selenium concentration and immune function in weaning pigs. Journal of Anim. Sice. and Biot., 5: 46.

Cheah, Y. and Yang, W. (2011). Functions of essential nutrients for high-quality spermatogenesis. Adv. Biosci. Biotechnol., 2: 182-197.

Chenoweth, P.C. (1981). Libido and mating behavior in bulls, boar and rams. A review. Theriogenology, 16: 155 -177.

Cortinhas, C.S.; Junior, J.E.; Naves, J.D.; Porcionato, M.A.; Silva, L.F.; Renno, F.P. and Santos, M.V. (2012). Organic and inorganic sources of zinc, copper and selenium in diets for dairy cows: intake, blood metabolic profile, milk yield and composition. R. Bras. Zootec., 41(6): 1477 – 1483.

Curry, M.R and Watson, P.F. (1994). Osmotic effects on ram and human sperm membrane in relation to thawing injury. Cryobiology, 31: 39 -46.

Dhami, A.J.; Sahni, K.L.; Mohan, G. and Tripathi, R.P. (1994). Comparative evaluation of initially static and motile semen ejaculate from Friesian and Murrah buffalo bulls for physico-morphological, biochemical, enzymatic and mineral constituents of seminal plasma. Indian J. Anim. Sci., 64: 926-932.

Doumas, B.; Watson, W. and Biggs, H. (1971). Albumin standards and measurements of serum with bromocresol green. Clin. Chem. Acta., 31: 87 - 96.

Duncan, D. B. (1955). Multiple range and multiple F. Test. Biometrics, 11: 1 – 42.

Ekins, R.P. (1984). Free hormones in blood. Concept and measurements. J. Clin. Immunoassay, 7: 163 – 180.

El-Fouly, M.M.; Fawzi, A.F.A.; Fergany, A.H. and El-Baz, F.K. (1984). Micronutrients status in crop in selected area in Egypt. Commune. Soil Sci. Plant. Anal., 15: 1175 – 1189.

El-Gohary, E.S.; Gabr, Sh. A. and El-Saadany, S.A. (2008). Sexual desire, semen quality and sperm fertility of Zaraibi bucks fed medicinal herbs and plants as feed additives. Egypt. J. Basic. Appl. Physiol., 7 (2): 419 – 435.

El-Hawary, A.F.E. (2010). Reducing perioxidation process in buffalo semen in response to some antioxidant treatments. Ph.D. Thesis, Fac. Agric., Mansoura Univ, Egypt.

El-Hawary, A.F.;El-Gohary, E.S.H.; Gabr, A.A.; El-Kishk, M.A.M. and El-Malky, O.M. (2017). Frozen-thawed bull spermatozoa quality and fertility rate added with seleno-zinc methionine. 1- Effect of zinc methionine and seleno-methionine on freezability and fertility of bull semen. J. of the Egypt. vet. Med. Assoc., 17(2): 259 - 275.

El-Hawary, A. F. A. and Abd El-Hady, M. A .A. (2018). Improving the Immune and Health Status and Blood Constituents of Egyptian Buffaloes and their Offspring in Response to Treating Dams with Seleno-methionine and Levamisole. J.Animal and Poultry Prod., Mansoura Univ., 9 (7): 305 – 314.

Elkomy, A.E.' El-seiby, M.E. and Kamel, K.L. (2008). Comparative study of semen quality and free amino acid content in seminal plasma between high and low motile sperm rabbit bucks. Egypt. Poult. Sci., 28: 663 – 649.

El-Nagar, H. A.; Hammed, M.E.R.; Hemida, A.R. and Gabr, A.A. (2021). Productive performance of Egyptian buffalo bulls administrated with N-Acetylcyteine, L- carnitine or their combination. Egyptian J. Nutrition and Feeds. 24(2): 199 -209.

El-Sheltawi, M.A.; Mary Abdel-Malak, G.; Abdel-Malak, G. and Khalifa, T.A. (1999). Impact of zinc and tocopherol on functional competence of cryopreserved buffalo spermatozoa. Assiut Vet. Med. J., 42: 291 – 309.

El-Shennawy (2013). A study on Friesian bulls semen. M. Sc. Thesis, Fac. Agric., Mansoura University, Egypt.

El-Siefy, E.M.E. (2004). Reproductive aspects on effect of selenium and vitamin E on semen quality and fertility of Egyptian Buffalo bulls. Ph. D. Thesis, Fac. Of Agric., Kafr El-Sheikh, Tanta Univ., Egypt.

El-Tohamy, M.M.; Kotp, W.S.; El-Nattat, S.A. and Mohamed, A.H. (2012). Semen characteristics and oxidative/antioxidative status in semen and serum of male rabbits supplemented with antioxidants during heat stress. Iranian Journal of Applied Animal Science, 2 (2): 175-183.

Foulkes, J. A. and Watson, P.A. (1975). Hyaluronidase activity in seminal plasma as a method of assessing bull sperm integrity. J. Repord. Fertil., 43: 349 -353.

Fukada, T.; Yamasaki, S.; Nishida, K.; Murakami, M. and Hirano, T. (2011). Zinc homeostasis and signaling in health and disases. J. Biol. Inorg. Chem., 16: 1123 – 1134.

Gabr, A. A. (2009). Assessment of in vitro and in vivo fertilizing capacity of buffalo sper treated with various cryoprotectents. Ph. D. Thesis, Fac. of Agric. Mansoura Univ. Egypt.

Gabr, S. A. A. (2000). Physiological aspects of zinc status and its effect on buffalo bulls semen quality. M. Sc. Thesis, Fac. of Agric., Kafr El-Sheikh, Tanta Univ., Egypt.

Ghorbani, A.; Moeini, M.M.; Souri, M. and Hajarian, H. (2018). Influences of dietary selenium, zinc and their combination on semen characteristics and testosterone concentration in mature rams during breeding season. Journal Of Applied Animal Research, 46 (1):813–819.

Hassan, E.H.; Farghaly, M.M. and Solouma, G.M. (2016). Effect of zinc supplementation from inorganic and organic sources on nutrient digestibility, some blood metabolites and growth performance of growing buffalo calves. Egypt. J. Nutr. And Feeds,19 (1): 37 -46.

Helper, O. E. (1966). Manual of clinical laboratory methods. Thomos Spring Filed. Illinois.

Hempe, J.M. and Cousins, R.J. (1989). Effect of EDTA and zinc methionine complex on zinc absorption by rat intestine. J. Nutr. 119: 1179 -1187.

Hussein, Y.S. (2018). Cryoprotective efficacy of local Friesian bull's spermatozoa using tris-extender enriched with natural or synthetic antioxidants. Egyptian J. Anim. Prod., 55(1): 1 - 6.

Imam, S.; Ansari, M. R.; Kumar, R.; Mudgal, V, V.; Arshney, V. P. and Dass, R. S. (2009). Effect of inorganic and organic zinc supplementation on serum testosterone level in Murrah buffalo (*Bubalus bubalis*) bulls. *Indian Journal of Animal Sciences*, 79 (6): 611–614.

Kala, A.; Dass, R.S. and Garg, A.K. (2016). Effect of zinc supplementation from inorganic and organic sources on blood biochemical, serum enzyme, minerals, vitamin and hormones in kids. Animal Nutrition and Feed Technology, 16: 129-142.

Kassab, A.Y. and Mohammed, A.A. (2014). Effect of vitamin E and selenium on some physiological and reproductive characteristics of sohagi ewes. Egyptian Journal Nutrition and Feeds., 17(1): 9 – 18.

Khalifa, H.H.; Ashour, A. M.; Youssef, M.M. and Ghoneim, M.M. (2011). Physiological response, blood parameters and reproductive performance of buffalo cows treated with zinc methionine and niacin under cooling system during summer season in Egypt. J. Animal and Poultry Prod., Mansoura Univ., 2 (2): 1 – 12.

Khan, A.A. (1994). Sexual behavior of the male camel (Camelus dromedaris) and some studies semen. M.Sc. Thesis, Bikaner Univ. Udaipur, India.

Kobadie, O. M. I. (2016). Study on freezing buffalo semen using different antioxidants. M.Sc. Thesis, Fac. Agric., Tanta Univ., Egypt.

Koracevic, D.; Koracevic, G.; Djordjeic, V.; Andrejevic, S. and Cosic, V. (2001). Method for the measurement of antioxidant activity in human fluide. J. Clin. Pathol., 54: 356 -361.

Kulkarni, B.A.; Dhamds, S.C. and Patankar, D.D. (1996). Seminal plasma protein profiles of Murrah and surti buffalo bulls. Ind. J. Anim. Sci., 66 : 900 – 903.

Kumar, P.; Yadav, B. and Yadav, S. (2013). Effect of zinc and selenium supplementation on antioxidative status of seminal plasma and testosterone, T4 and T3 level in goat blood serum. Journal of Applied Animal Research, 41(4): 382-386.

Lucky, Z. (1977). Methods of the diagnosis of ish diseases. Ameruno Publishing Co., PVT, Ltd. New Delhi., Bomby, New York.

Mamdouh, M.S.M. (2000). Studies on some factors affecting reproductive performance of bovine. Ph.D. Thesis, Fac. Vet. Med., Zagazig Univ., Cairo, Egypt.

Mandal, G. P.; Dass, R. S.; Garg, A. K.; Varshney V. P, and Mandal, A. B. (2008). Effect of zinc supplementation from inorganic and organic sources on growth and blood biochemical profile in crossbred calves. Journal of Animal and Feed Sciences, 17: 147–156.

NRC (2001). Nutrient Requirement in Dairy Cattle. 7th Ed., Nat. Acd. Press, Washington, D.C.

Osama , M. A. 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.

Pankaj, K., Brijesh, Y. and Sarvajeet, Y. (2014). Effect of zinc and selenium supplementation on semen quality of Barbari buck. Indian J. Anim. Res., 48(4): 366 – 369.

Rashid A and Ryan J. (2008). Micronutrient constraint to crop production in the near east potential significance and management strategies. micronutrient deficiencies in global crop production. pp. 149-80.

Richard, M.J.; Portal, B.; Meo, J.; Coudray, C.; Hadjian, A. and Favier, J. (1992). Malondialdehyde kit evaluated for determining plasma and lipoprotein fractions that react with thiobabituric acid. Clin. Chem., 38: 704 – 709.

Safarinejad, M. R. and Safarinejad, S. (2009). Efficacy of selenium and/or N-acetyl-cysteine for improving semen parameters in infertile men: a double-blind, placebo controlled, randomized study. J Urol., 181 (2): 741 – 751.

Salisbury, G.W.; Van Demark, N.L. and Lodge, J.R. (1978). Physiology of Reproduction and Artificial Insemination of Cattle. W.H. Freeman and Company, San Francisco, USA.

Sanchez-Gutierrez, M., Garcia-Montalova, E.E. and Izquierd-Vega, G.A. (2008). Effect of dietary selenium deficiency on the in vitro fertilization ability of mice spermatozoa. Cell Biology and Toxicology, 24: 321 – 329.

Shi, L.; Zhang, C.; Yue, W.; Shi, L.; Zhu, X. and Lei, F. (2010). Short term effect of dietary selenium enriched yeast on semen parameters, antioxidant parameters and Se concentration in goat seminal plasma. Anim Feed Sci Tech., 157 (1-2):104-108.

Singh, P.; Sengupta, B.P. and Tripathi, V.N. (2000). Effect of supplemental amino acids feeding a sexual behavior, quality and freezability of buffalo bull semen. Buff. J., 1: 41-46.

Smith, O.B. and Akinbamizo, O.O. (2000). Micronutrients and reproduction in farm animals. Animal Reproduction Sci., 60-61: 549 – 560.

Solomons, N.W. (1998). Mild human zinc deficiency produces an imbalance between cell-mediated and humoral immunity. Nutr. Rev. 56: 27 -28.

SPSS (2013). SPSS user Guide: Statistics SPSS.

Tabl Ghada, A. ; El-Desouki Nabila, I.; Zeidan, A. E. B., Tag El-Deen M. A. and Kamel Rabab, A. (2012). Effect of anthelmintics drugs on biochemical characteristics of bull Friesian frozen semen.Proc. 7th Int. Con. Biol. Sci. (Zool) 203 – 213.

Taha, T.A.; Abdel-Gawad, E.I. and Ayoub, M.A. (2000). Monthly variation in some reproductive parameters of Barki and Awassi rams throughout one year under subtropical condition. 2: Biochemical and enzymatic properties of seminal plasma. Anim. Sci., 71: 325 - 328..

Ullrey, D. E. (1980). Regulation of essential nutrient additions to animal diets (Selenium-A model case). J. Anim. Sci., 51: 645.

Watson, P.F. (1975). Use of a Giemsa stain to detect change in acrosomes of frozen ram spermatozoa. Vet. Rsc., 97: 12 – 15.

Weichselbaum, T. E. (1946). An accurate and rapid method for the determination of proteins in small amounts in blood serum and plasma. Am. J. Clin. Pathol.,16: 40-47.

Young, S.S.; Eskenazi, B.; Marchetti, F.M.; Block, G. and Wyrobek, A.J. (2008). The association of folate, zinc and antioxidant intake with sperm aneuploidy in healthy non-smoking men. Hum. Reprod., 23: 1014 – 1022.

Zeidan, A. E. B.; Abd El-Kariem, M.A. and Mohamed, M.M. (2004). Semen quality, acrosomal status and ability of bovine spermatozoa to penetrate cervical mucus during different freezing processes and thawing rates. 3rd Int. Conf. on Anim., Poul. & Fish Prod. And Health In Semi-Arid Areas. Al-Arish, North Sinai, pp. 81 -97.

Zeweil, H.S; Zahran, S.M.; Ebeid, T.A.; El-speiy, M.E.; Yassmine, M. El- Gindy and Abd Elaal, M.A. (2017). Effect of fasting regimen and dietary zinc supplementation on hematological parameters, hormonal profile and antioxidant properties in males of growing rabbits. Egypt. J. Nutr. And Feeds. 20(1): 67 -79.

الأداء التناسلي لطلائق الفريزيان المعاملة بالسيلينوميثيونين، الزنك ميثيونين أو كليهما

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الغرض من هذه الدراسة هو دراسة تقييم تجريع طلائق الفريزيان ببعض مضادات الأكسدة العضوية مثل السيلينوميثيونين و الزنك ميثيونين ، منفردين أو معاً علي بعض سمات مقاييس الدم و الرغبة الجنسية وجودة السائل المنوي الطازج والسائل المنوي المجمد و المسال علي معدلات إسالة مختلفة و كذلك القدرة الإخصابية للحيوانات المنوية للطلائق. تم توزيع 16 طلوقة فريزيان علي أربع مجموعات بكل منها 4 طلائق ، غذيت جميع الطلائق علي العليقة الأساسية ، المجموعة الأولي تركت بدون معاملة (ضابطة) ، و المجموعة الثانية و الثالثة و الرابعة تم تجريعها ب 0.3 ملليجرام سيلينوميثيونين و 4 ملليجرام زنك ميثيونين و 0.15 ملليجرام سيلينوميثيونين بالإضافة إلي 2 ملليجرام زنك ميثيونين من المادة الجافة / طلوقة / يوميا علي التوالي لمدة 150 يوماً. أوضحت النتائج أن الطلائق التي تم تجريعها بخليط من السيلينوميثيونين مع الزنك ميثيونين سجلت أفضل القيم لكل من الصفات الهيماتولوجية و العد التفريقي لكرات الدم البيضاء و مستوي هرمون التستوستيرون في الدم و الفركتوز الأولي في بلازما السائل المنوي الطازج و الرغبة الجنسية بالإضافة إلي الصفات الطبيعية للسائل المنوي الطازج و السائل المنوي المجمد و المسال علي معدلات إسالة مختلفة مقارنة بطلائق المجموعة الضابطة أو المجموعات الأخرى. لوحظ أيضا أن أفضل جودة و قابلية للتجميد و خصائص لبلازما السائل المنوي و مقدرة إخصابية للحيوانات المنوية قدرت عند إسالة السائل المنوي المجمد علي درجة حرارة 55º م لمدة 15 ثانية (سريع).

خلصت هذه الدراسة إلي أن تجريع طلائق الفريزيان المستخدمة في التلقيح الإصطناعي بـ 0.15 ملليجرام سيلينوميثيونين بالإضافة إلي 2 ملليجرام زنك ميثيونين كمضادات أكسدة عضوية لمدة 150 يوم تعتبر إستراتيجية مناسبة لتعزيز الحالة المناعية والصحية للطلائق ولتحسين جودة السائل المنوي الطازج و قابليته للتجميد و الإسالة بالإضافة إلي المقدرة الإخصابية للحيوانات المنوية خاصة عند إسالتها علي درجة حرارة 55º م لمدة 15 ثانية (إسالة سريعة).