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Selenium and Zinc as Supplements to Extenders Frozen Semen for Improving Sperm Characteristics during Cryopreservation

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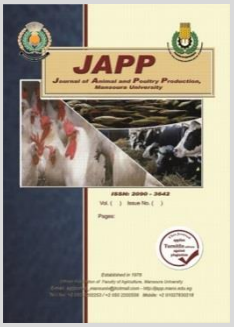

ABSTRACT

Aim of this study is to evaluate the effect of adding different levels of Zinc and Selenium on extender as antioxidants to improve Friesian-bull semen quality after cryopreservation. Semen was collected, pooled, and extended with five treatment extenders, including control without (E1), 0.3 mg/100ml Zn (E2), 0.6 mg/100ml Zn (E3), 0.2 mg/100ml Se (E4), and 0.5 mg/100ml Se (E5). Semen was evaluated for visual parameters after dilution, equilibration, and thawing. Also, semen was evaluated by CASA only after thawing. Results showed that there are no effects of all additives on sperm characteristics after dilution and thawing. Only visual progressive motility percentage increased (P<0.05) post-equilibration by E5. Percentages of acrosome integrity were enhanced by E4 and E5. Semen analysis by CASA revealed that non-motility, and total and head abnormalities percentages were decreased (P<0.05), while vitality, and total and rapid progressive motility percentages were increased (P<0.05) by E5. Dynamic sperm parameters straightness and wobble) were increased (P<0.05) E5. In conclusion, adding selenium at a level of 0.5 mg/100ml to extender of cryopreserved Friesian-bull semen ha beneficial effects on maintaining sperm parameters and improved sperm freezability. This may be used as a tool for improving cryopreserved semen in artificial insemination centers.

Keywords: Friesian semen, zinc, selenium, cryopreservation, sperm dynamics.

INTRODUCTION

Artificial insemination is a valuable tool in genetic improvement programs and a widely used as a breeding technique in farm animals (Sansone *et al*., 2000). Semen processing and cryopreservation cause considerable damage to the sperm DNA, motility apparatus, plasma membrane, acrosomal cap, leakage of intracellular enzymes, and reduced fertility (Rasul *et al*., 2001; Dhami and Kodagali, 1990; Chohan *et al*., 2006; Guthrie and Welch, 2006). Cryopreservation of semen induces the oxidative stress on function and structure of spermatozoa (Chatterjee and Gagnon, 2001). The production of excessive reactive oxygen species (ROS) damages the spermatozoa by lipid peroxidation in the sperm cell during freezing (Arabi *et al*., 2001; Hashem *et al*., 2013). Rising levels of ROS like superoxide, hydroxyl, and hydrogen peroxide significantly affect the semen quality parameters like motility, morphology, sperm functions and sperm DNA (Hellstrom *et al*., 1994).

Antioxidants are the agents, which break the oxidative chain reaction eliminating, taking up, or reducing the formation of ROS (Bansal and Bilaspuri, 2011) and thereby reduces the oxidative stress (Miller *et al*., 1993; Kumar and Mahmood, 2001). The antioxidants check the chemical breakdown of the substrate resulting from oxidation and neutralize the free radicals thus reducing the risk of damage to spermatozoa during cryopreservation (Strzezek, 2002; Pen˜a *et al*, 2003; Roca *et al*, 2004). Antioxidants may be preventive antioxidants (metal chelators or binding proteins, such as lactoferrin and transferring), which prevent the formation of ROS, or scavenging antioxidants, like vitamins C and E, which removes the ROS that is already present (Lampiao, 2012).

Selenium (Se) is found in the earth's crust in association with metals and in traces in water. It is a necessary trace nutrient for growth and development of humans and animals. In rat, mice, chicken, pig, sheep, and cattle (Baiomy *et al*., 2009), Se insufficiency has been associated with reproductive complications and decreased sperm quality. It constitutes a necessary part of glutathione peroxidase, an enzyme responsible for protecting cell internal structures from free radicals. It is considered an excellent antioxidant for cellular membrane lipids (Gutierrez *et al*., 2008). The significance of Se is pronounced from the fact that Se supplementation proves the better storage along the less release of lipids from the sperm cell during long time storage (Dimitrove *et al*., 2007). Several studies have reported the effects of antioxidants on semen extenders during the cryopreservation and significant correlation between Se level in the seminal plasma and sperm integrity (Watanabe and Endo, 1991; Noack-Filler *et al*., 1993). Selenium is an essential micronutrient which generally acts as a co-factor and is present in some enzymatic structures called selenoproteins in vivo (Sarkar *et al*., 2015; Zeliha *et al*., 2015). Selenium at a level (2 µg /mL) can improve the quality of fresh and frozen semen of buffalo bull, mice, human, ram, bovine and other species (Bilodeau *et al*., 2001). However, there is a very narrow margin between activity and toxicity of selenium (Lanctot *et al*., 2017).

Zinc (Zn) is one of the important trace elements in the body deficiency of which causes infertility in most animals due to disorders of testes development and spermatogenesis (Massanyi *et al*., 2004). It plays basic role in membrane

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stability and physical properties of the attached fibers, sperm tail morphology and sperm motility. Lack of Zn may increase oxidative damage, resulting in poor sperm quality (Colagar *et al*., 2009). Zinc in the sperm reacts to tight dense fiber that reacts with sulfhydryl groups and prevents oxidation (Henkel *et al*., 2001). Some in vivo evidence suggests that Zn acts as in vivo as a superoxide cleaner produced by incomplete spermatozoids or leukocytes.

Other tests have shown that Zn can clean up radicals induced by various factors, including ionizing radiation, and reduce malondialdehyde (MDA) levels, so it is known as a highly antioxidant (Dani and Dhawan, 2005). Supplementation of Zn leads to improve fertility in Zn deficient animals by increasing concentration and motility of spermatozoa and sperm membrane integrity (Karaji *et al*., 2014). The high concentration of Zn may depress oxygen uptake (cell respiration) by sperm cells and influence the sperm motility. Zinc antioxidant capacity has been reported by many authors (Powell *et al*., 2000; Dissanayake *et al*., 2006). Buffalo semen samples with higher seminal plasma Zn content had higher sperm motility and viability, and lower abnormal morphology (Alavi-Shoushtari *et al*. 2009).

Aim of this study was to evaluate the effect of adding two levels of zinc in comparing with selenium, as antioxidants, to extender on sperm characteristics of cryopreserved semen of Friesian bulls.

MATERIALS AND METHODS

This study was conduct in Sakha Animal Production Research Station, Animal Production Research Institute (APRI), Agricultural Research Center.

Semen was collected from 3 Friesian bulls, early in the morning, twice a week for seven weeks, using artificial vagina. The ejaculates were placed in water bath at 37°C and transported to the laboratory of International Livestock Management Training Center (ILMTC) to semen evaluation. Initial motility was assessment immediately after collection, and ejaculates having over 70% percentage were chosen for further evaluation. On day of semen collection, semen was pooled and diluted with bovine base-extender OptiXcell (IMV technologies, France) supplemented with different antioxidants at a level of 1: 15.

In this study, semen was diluted with OptiXcell supplemented with 0 (E1), 0.3 mg Zn/100ml (E2), 0.6 mg Zn/100ml (E3), (Zinc sulphate (monohydrate) extra pure, Oxford).0.2 mg Se/100ml (E4), and 0.5 mg Se/100ml (E5), (Selenium DIOXIDE (Sublimed) 99% Extra Pure, Oxford).

After dilution of the semen with different supplementation at 37ºC, then cooled to 5ºCfour hours as an equilibration period (Andrabi *et al.*, 2006). After equilibration, semen straws of 0.25 mL capacity were packed and placed horizontally on rack at 5 cm higher on the liquid nitrogen vapors for 10 minutes. The frozen straws of semen were plunged into liquid nitrogen container and stored on this temperature for one month until further evaluation.

Semen extended with each supplement was evaluated in post-dilution, and post-equilibration, and post-thawing at 37oC for 20 s. According to Asr *et al*. (2011), percentage of progressive motility was measured by putting small drop of extended semen on warmed slide. This slide was enclosed with the help of cover slip to examine at 40x of phasecontrast microscope. Percentages of viability and morphological abnormalities of spermatozoa for at least 100 spermatozoa, were done using Eosin (0.5%)- Nigrosin (0.1%) staining mixture. Dead cells were stained by the Eosin (Barbas, and Mascarenhas, 2009).

In post-thawed semen, semen was evaluated by CASA (SPERMOLAB®, Cairo,Egypt) for sperm motility parameters including percentages of total sperm motility (TMS %), progressive sperm motility (PMS %), non-progressive sperm motility (NPMS %), immotile sperm (MMS %), rapid progressive motility, and slow progressive motility. Percentages of sperm vitality, normal sperm, and sperm abnormalities (head, neck, and tail) was also automatically determined by CASA and reported. In addition, sperm kinetic parameters, involving curve linear velocity (VCL), straight linear velocity (VSL), and average path velocity (VAP) were estimated. Linearity (LIN), straightness (STR), and wobble (WOB) as ratios of the velocity parameters (VSL/VCL%, VSL/VAP%, and VAP/VCL%, respectively) were also calculated.

In post-thawed semen, the spermatozoa acrosomal integrity was monitored by mixing 500 µL of semen with 50 µL of 1% formaldehyde citrate in a test tube. A single drop of semen was observed below stage contrast microscope at 1000X as described by Asr *et al*. (2011) to count two hundred sperms with acrosome abnormalities. membrane integrity assessed by the hypo-osmotic swelling test (HOST), as described by Jeyendran *et al*. (1984). In brief, the hypo-osmotic solution (osmotic pressure ≡150 mOsmol kg-1, Osmomat 030; Gonotec, Berlin, Germany) was prepared by dissolving 0.73 g sodium citrate and 1.35 g fructose in 100 mL of distilled water. Hypo-osmotic solution (500 µL) was mixed with 50 µL of semen and incubated at 37 ˚C for 40 min. After incubation, a drop of semen sample was examined under a phase-contrast microscope (Model BX41, Olympus Corp., Tokyo, Japan) (400×) and 200 spermatozoa were counted in at least five different fields for their swelling charact-erized by coiled tail indicating intact plasma membrane.

Data were statistically analyzed by one way-ANOVA using SAS program (SAS, 2004) to test the effect of supplementation. The significant differences among means were set at $P<0.05$ according to Duncan (1955). All percentage values were transformed to arcsine values before the statistical analysis.

RESULTS AND DISCUSSION

Results

Post-diluted semen:

Visual sperm characteristics:

The results in Table 1 show that adding both levels of Zinc or Selenium didn't effect on the percentage of visual progressive motility, livability, and abnormality of spermatozoa in post-diluted semen as compared to control. Although the percentage of progressive motility was affected ($P<0.05$) by extender treatment, but the differences were significant between E3 and E5.

Table 1. Sperm characteristics as affected by adding zinc and selenium in extender of Friesian bull post- diluted semen.

Sperm Parameter (%)	E1 (control)	E2 (0.3 mg/100ml Zn)	E3 (0.6 mg/100ml Zn)	E4 (0.2 mg/100ml Se)	E5 (0.5 mg/100ml Se)
Progressive motility	71.67±1.67^{ab}	73.33±1.67^{a}	66.67±1.67^{b}	71.67±1.67^{ab}	73.33±1.67^{a}
Livability	72.67±0.89	76.00±1.53	68.67±2.91	72.33±2.19	73.00±2.89
Total abnormality	17.67±2.19	17.33±1.33	17.00±1.01	18.67±0.667	15.33±1.45

Means in the same row with different superscript (a, b) differ significantly (P<0.05).

Post-equilibrated semen:

Visual sperm characteristics:

Post-equilibration, sperm progressive motility increased (P<0.05) only with E5, but did not differ significantly with E2. However, sperm livability percentage increased in E5 compared with E1, but the differences were not significant. Moreover, there were no significant differences in sperm abnormality percentage (Table 2).

Table 2. Sperm characteristics as affected by adding zinc and selenium in extender of Friesian bull post- equilibrated semen.

Sperm parameter (%)	E1 (control)	E2 (0.3 mg/100ml Zn)	E3 (0.6 mg/100ml Zn)	E4 (0.2 mg/100ml Se)	E5 (0.5 mg/100ml Se)
Progressive motility	61.67±1.67^{b}	63.33±1.67^{ab}	61.67±1.67^{b}	61.67±1.67^{b}	68.33±1.67^{a}
Livability	66.00±1.53^{ab}	68.00±1.53^{ab}	62.67±0.667^{ab}	62.00±1.53^{b}	68.67±2.91^{a}
Total abnormality	21.00±2.52	17.67±.882	22.00±1.53	20.00±.577	19.67±1.45

Means in the same row with different superscript (a, b) differ significantly (P<0.05).

Post-thawed semen:

Visual sperm characteristics:

Percentage of sperm progressive motility and livability decreased (P<0.05) only with E3 as compared to E1 and other treatments. But did not differ significantly with E2. Although the percentage of sperm abnormality was affected (P<0.05) by extender treatment, but the differences were significant between E3 and E5 (Table 3).

Results presented in Table 4 revealed that only selenium treatments (E4 and E5) had remarkably positive influence on the percentage of acrosome integrity as compared to control (E1) and the other groups. Treatments in E2, E4, and E5 had no effect on plasma membrane integrity, but E3 showed adverse effect on post-thawed semen quality in term of decreasing membrane integrity percentage.

Table 3. Sperm characteristics as affected by adding zinc and selenium in extender of Friesian bull post- thawed semen.

Sperm parameter (%)	E1 (control)	E2 (0.3 mg/100ml Zn)	E3 (0.6 mg/100ml Zn)	E4 (0.2 mg/100ml Se)	E5 (0.5 mg/100ml Se)
Progressive motility	46.67±1.67^{ab}	48.33±1.67^{a}	16.66±1.67^{c}	41.67±1.67^{b}	50.00±2.89^{a}
Livability	53.00±2.00^{ab}	56.00±1.15^{a}	24.00±2.08^{c}	49.33±1.20^{b}	58.67±2.33^{a}
Total abnormality	25.00±2.08^{ab}	25.00±1.52^{ab}	28.00±1.00^{a}	25.33±1.20^{ab}	22.66±1.45^{b}

Means in the same row with different superscript (a, b, c) differ significantly (P<0.05).

Table 4. Integrity of sperm plasma membrane and acrosome as affected by adding zinc and selenium in extender of Friesian bull post-thawed semen.

parameter	E1 (control)	E2 (0.3 mg/100ml Zn)	E3 (0.6 mg/100ml Zn)	E4 (0.2 mg/100ml Se)	E5 (0.5 mg/100ml Se)
Acrosome integrity (%)	55.33±0.667^{b}	59.33±1.33^{ab}	54.67±1.76^{b}	62.00±0.00^{a}	62.67±3.53^{a}
Membrane integrity (%)	54.67±1.76^{a}	57.33±3.53^{a}	46.67±2.40^{b}	52.66±1.33^{ab}	52.00±1.155^{ab}

Means in the same row with different superscript (a, b) differ significantly (P<0.05).

CASA sperm characteristics:

Sperm motility:

Results in Table 5 revealed that E5 had significantly positive effect on enhancing the percentages of vitality, progressive motility, and rapid progressive motility compared with control extender. Each of E2 and E4 did not alter sperm characteristics in comparing with E1, except for non-progressive motility percentage. On the other hand, E3 showed negative effects on most of sperm characteristics.

Table 5. Sperm vitality and motility examined by CASA as affected by adding zinc and selenium in extender of Friesian bull post- thawed semen.

Sperm parameter (%)	E1 (control)	E2 (0.3 mg/100ml Zn)	E3 (0.6 mg/100ml Zn)	E4 (0.2 mg/100ml Se)	E5 (0.5 mg/100ml Se)
Vitality	89.13±1.39^{b}	87.31±2.97^{b}	56.33±6.53^{d}	71.20±6.33^{c}	98.76±1.89^{a}
Total motility	74.18±1.09^{ab}	74.47±2.19^{ab}	46.31±6.29^{c}	63.49±4.99^{b}	86.44±1.45^{a}
Non-motility	25.82±1.09^{cb}	25.53±2.19^{cb}	53.69±6.29^{a}	36.50±4.99^{b}	13.57±1.45^{c}
Progressive motility	54.04±1.82^{b}	57.26±1.67^{b}	25.99±1.48^{c}	51.55±3.90^{b}	72.20±1.18^{a}
Non-progressive motility	20.14±1.21^{a}	17.21±0.580^{ab}	20.32±4.82^{a}	11.94±1.33^{b}	14.24±0.272^{ab}
Rapid progressive motility	35.70±1.44^{b}	35.33±4.39^{b}	14.51±1.68^{c}	29.09±3.91^{b}	57.98±2.54^{a}
Slow progressive motility	18.33±2.45^{ab}	21.92±3.05^{a}	11.48±1.78^{c}	22.46±0.131^{a}	14.22±1.67^{bc}

Means in the same row with different superscript (a, b, c, d) differ significantly (P<0.05).

Sperm abnormalities:

Table 6 revealed that E5 enhanced (P<0.05) post-thawed semen quality by declining total sperm abnormality and abnormal head percentages to the lowest values as compared to E1 and other treatments. On the other hand, E3 showed an opposite trend to E5. However, the differences among treatments and control regarding the percentage of abnormality in neck and tail were not significant.

Table 6. Sperm morphological abnormalities by CASA as affected by adding zinc and selenium in extender of Friesian bull post- thawed semen.

Sperm parameter (%)	E1 (control)	E2 (0.3 mg/100ml Zn)	E3 (0.6 mg/100ml Zn)	E4 (0.2 mg/100ml Se)	E5 (0.5 mg/100ml Se)
Total abnormality	55.23±2.60^{b}	56.40±4.26^{b}	68.60±0.451^{a}	64.43±0.775^{ab}	37.21±5.29^{c}
Abnormal head	53.33±2.91^{b}	54.85±4.72^{ab}	64.03±1.64^{a}	62.05±1.21^{ab}	34.52±3.60^{c}
Abnormal neck	26.19±6.04	25.92±3.48	24.18±2.92	39.51±3.41	20.58±9.53
Abnormal tail	25.47±6.93	19.63±3.74	13.40±3.30	25.96±16.60	19.65±10.25

Means in the same row with different superscript (a, b, c) differ significantly (P<0.05).

Dynamic sperm parameters:

According data in Table 7, in comparing with E1, the most remarkable effects were observed for E5, in terms of decreasing VCL, VAP, and WOB, and increasing STR. However, E2 and E3 showed pronounced effect on WOB, but VAP was decreased by E2, while STR was increased by E3. E4 showed effects similar to E1.

Table 7. Post thawed Influence by adding two levels of zinc and selenium in extender on kinetic parameters of Friesian bull's semen.

Sperm parameter (%)	E1 (control)	E2 (0.3 mg/100ml Zn)	E3 (0.6 mg/100ml Zn)	E4 (0.2 mg/100ml Se)	E5 (0.5 mg/100ml Se)
VCL	22.69±2.87^{a}	15.12±0.914^{ab}	19.83±2.05^{ab}	19.90±4.56^{ab}	12.23±1.64^{b}
VSL	10.73±1.13	6.66±0.554	9.81±1.43	11.25±4.12	5.46±1.09
VAP	26.41±4.93^{a}	12.70±1.41^{bc}	15.87±1.01^{abc}	21.19±6.11^{ab}	8.84±1.26^{c}
LIN	47.65±2.04	44.18±3.03	48.98±2.33	42.84±3.83	44.65±5.75
STR	42.03±4.10^{b}	53.60±6.38^{ab}	61.34±6.45^{a}	39.31±4.19^{b}	61.28±5.57^{a}
WOB	114.8±7.42^{a}	83.51±4.91^{bc}	80.89±5.26^{c}	111.97±17.7^{ab}	72.33±2.88^{c}

Means in the same row with different superscript (a, b, c) differ significantly (P<0.05).
VSL, straight linear velocity (µm/s); VCL, curve linear velocity (µm/s); VAP, average path velocity (µm/s); LIN, linearity; STR, straightness; WOB, wobble

Discussion

Improvement in artificial insemination programs could be obtained by preserve sperm motility and fertility which affected negatively by steps of cryopreservation processing. Cryopreservation processing (cooling and freeze-thawing) loads a stress in both physical and chemical of sperm membrane (Chatterjee and Gagnon, 2001). Oxidative stress leads to defect in physiological characteristics of spermatozoa (Bilodeau *et al*., 2001). Previous studies have evaluated the effective of antioxidant additives supplementation in semen extender on the preservation of spermatozoa (Beconi *et al*., 1993; Szcześniak-Fabiańczyk *et al*., 2003).

The present study aimed to evaluate the effect of adding different levels of Zn and Se, as antioxidants, in extender on sperm characteristics examined visually and by CASA after dilution, equilibration, and freezing-thawing. At first, different levels of Zn and Se were added on fresh-diluted semen to evaluate which the proper level had the positive effect on sperm characteristics after cryopreservation. The effect of Zn and Se levels on sperm parameters after dilution and thawing was not significant as compared to the control extender. These results go along with Nair *et al*. (2006) or Pratt *et al*. (1980), who revealed no effect was recorded by adding Se before cryopreservation on sperm characteristics. Also, several authors showed the same results in ram and bovine spermatozoa (Pratt *et al*., 1980; Siegel *et al*., 1980; Alabi *et al*., 1985; Seremak *et al*., 1999;). Meanwhile, extender with 0.5 mg/100ml of Se (E5) improved (P<0.05) visual sperm motility in post-equilibrated semen as compared to the control extender. These results are in agreement with other in vitro and in vivo studies in buffalo, ram, and bovine (Pratt *et al*., 1980; Siegel *et al*., 1980; Seremak *et al*., 1999; Dorostkar *et al*., 2012). Beside the positive enhancement in visual sperm progressive motility post-equilibration, plasma membrane integrity percentage was also improved (P<0.05) by E5 in post-thawed semen. These results may indicate a protection effect of Se from cold chock on sperm motility during equilibration.

In addition, sperm motility parameters measured by CASA, including progressive motility and rapid progressive motility significantly increased, while sperm immobility and slow progressive motility significantly decreased by E5. Also, sperm vitality increased significantly, while sperm total morphological and head abnormalities significantly decreased by E5. Dorostkar *et al*. (2012) showed similar results in buffalo bull semen. Pratt *et al*. (1980) reported that Se addition lead to increase the concentration of ATP utilized enzymes and revival pathway of spermatozoa which are assessed by motility and oxygen consumption of sperm, that lead to improvement of spermatozoa progressive motility. Acrosome integrity is important key to evaluate the active function of sperm membrane (Silva and Gadella, 2006). Some authors observed that Se had a potential protection of spermatozoa membrane in different species (Gutierrez *et al*., 2008; Dorostkar *et al*., 2012; Angrimani *et al*., 2017). Premature cryocapacitation of sperm with standard cryopreservation before freezing is related to the change in the acrosomal integrity and makes sperm incapable to fertilize egg (Sansores *et al*., 2011). The beneficial function of Se is on antioxidant system of cells and protects the cell by forming catalytic site for antioxidant enzymes e.g. Glutathione peroxidase (Alvarez and Storey,

1989). The active protection of Se may preserve spermatozoa from oxidative stress which produced by cryopreservation technique (Kempna *et al*., 2004).

In our study, adding 0.5mg/100ml Se in treatment extender showed an improvement in spermatozoa viability percentage. Others earlier authors, observed same results in buffaloes, rams, and boar (Sansores *et al*., 2011; Dorostkar *et al*., 2012; Anghel *et al*., 2010). The possibility of Se capacity to reduce lipid peroxidation rate is by reacting within chain during the oxidative stress metabolism (Beconi *et al*., 1993; Kadirvel *et al*., 2009; Khan *et al*., 2012).

In our study, Zn at a level of 0.6 mg/100ml show a reverse result as compared low level of Zn, Se levels, and control. High level of Zn may cause a remarkable decrease in sperm characteristics after freeze-thawed Friesian bull semen. These results are in agreement with Dorostkar *et al*. (2014), who revealed that Zn at levels 0.576 and 1.152 µg/L as a zinc sulphate, result in deleterious defects on sperm characteristics. Earlier reports suggested that the reasons of the negative effects of high levels of Zn are increased release of free Zn fraction and its subsequent uptake by spermatozoa (Carpino *et al*., 1998) and decrease in oxygen consumption, since high levels of Zn in semen impairs the oxygen consumption of spermatozoa (Sorensen *et al*., 1999). This elevated free Zn fraction may be accounted for lower sperm characteristics by E3.

Evaluating the semen of different extenders by CASA showed an association of improvement in motility parameters, vitality, acrosome integrity, and morphological abnormalities in E5 with kinetic sperm parameters such as LIN, STR, and WOB, which may indicate the positive impact of addition of Se at a level of 0.5 mg/100ml.

Based on the obtained results, it was supposed that the differences between in the effect of Zn and Se on sperm characteristics can be related to variation in levels of the supplements, different antioxidant capacity of each sperm in the testing stage, extender composition, and the interaction between type of extender and the supplementation. These factors may explain why both levels of Zn or Se at a low level did not improve the sperm characteristics.

CONCLUSION

Adding selenium at a level of 0.5 mg/100ml to extender of cryopreserved Friesian-bull semen ha beneficial effects on maintaining sperm parameters and improved sperm freezability. This may be used as a tool for improving cryopreserved semen in artificial insemination centers.

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الزنك و السيلينيوم كاضافات الى مخفف السائل المنوى المجمد لتحسين خصائص السائل المنوى بعد الحفظ بالتجميد

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تهدف هذه الدراسة إلى تقييم تأثير إضافة مستويات مختلفة من الزنك والسيلينيوم على مخفف السائل المنوى كمضادات للأكسدة لتحسين جودة السائل المنوي لطلائق الفريزيان خلال مراحل الحفظ المختلفة. تم جمع السائل المنوي وتخفيفه بخمس مخففات معاملة ، بما في ذلك المخفف الغير معامل (بدون) (م١) ثم مخفف معامل بتركيزين من الزنك التركيز الاول ٠,٣ جم/١٠٠ملليلتر (م٢) والتركيز الثانى ٠,٦ جم/١٠٠ ملليلتر (م٣) و مخفف معامل بتركيزين من السيلينيوم التركيز الاول ٠,٢ جم/١٠٠ ملليلتر (م٤) و التركيز الثانى ٠,٥ جم/١٠٠ملليلتر (م٥). تم تقييم خصائص الحيوانات المنوية بصريا بواسطة المجهر بعد التخفيف والموازنة والذوبان. أيضا ، تم تقييم خصائص المورفولوجية للحيوانات المنوية و الخصائص الحركية بواسطة CASA فقط بعد الذوبان. أظهرت النتائج عدم وجود تأثير لجميع المواد المضافة على خصائص الحيوانات المنوية بعد التخفيف والذوبان. زادت نسبة الحركة التقدمية البصرية (بواسطة المجهر فقط بعد الموازنة بواسطة E5. واظهرت النتائج وجود تحسن فى سلامة الاكروسوم بواسطة مخففات السيلينيوم (م٤ و م٥). أظهر تحليل السائل المنوي بواسطة CASA أن نسبة عدم القدرة على الحركة والشذوذ الكلي والشذوذ فى الرأس قد انخفضت ، بينما زادت نسب الحركة التقدمية الكلية والسريعة فى (م٥). تمت زيادة استقامة الحيوانات المنوية وتمايلها فى (م٥).الخلاصة :- اضافة السيلينيوم بمستوى ٠,٥ جم/١٠٠ملليلتر (م٥) الى مخفف السائل المنوى لطلائق الفريزيان يؤدى الى تحسين القدرة التجميدية للحيوانات المنوية خلال مراحل الحفظ المختلفة مما يمكن استخدامه لتحسين السائل المنوى المحفوظ فى مراكز التلقيح الاصطناعى.