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Effect of some Semen Extenders as a Natural Source of Antioxidants on Quality of Frozen Friesian Semen

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ABSTRACT

This study was designed to evaluate the possibility of using the optimal level of *Moringa oleifera* leaf aqueous extract (10, 15 or 20%) with 90, 85 or 80% saline (MOLE) as a semen extender of bull semen in comparing with three extenders, including Tris-egg yolk (TEY) without or with vitamin C at a level of 0.9 mg/ml (TEY+VC), and camel skim milk (CSM). Semen was collected from five Friesian bulls (350-450 kg LBW and 3-4 years old), twice a week for 5 weeks using artificial vagina. After ejaculation, mass motility was evaluated and only those of ≥70% were used in this study. Ejaculates were pooled, divided into 4 replicates and prepared for freezing. Semen was extended in TEY (control), TEY+VC, (CSM) and 15% MOE+85% saline (based on the best results of MOLE level). Semen was diluted, equilibrated at 5^{o}C (4 h) and placed in liquid nitrogen (LN) at -196^{o}C. Frozen semen stored for one month at least was warmed at 37^{o}C for 30 sec. Semen evaluation for percentages of individual motility (IM), live sperm (LS), sperm abnormality (SA), acrosomal damage (AD) and hypo-osmotic swelling test (sperm cells with curled tail, CT) was estimated in diluted, equilibrated and thawed semen, while percentage of head to head agglutination (HHA) was determined in post-thawed semen only. Activity of AST, ALT, LDH and total antioxidant activity were determined in seminal plasma of post-thawed semen. Conception rate (CR) was recorded for Friesian cows inseminated with semen frozen with the four types of extenders. Results showed that percentages of IM, LS, SA, AD and CT in post-diluted and post-equilibrated semen, beside all previous characteristics and percentage of HHA in post-thawed semen showed the best results (P<0.05) in CSM, moderate in 15% MOE and TEY+VC, and the poorest in TEY. Enzyme activities of AST, ALT and LDH in post-thawed seminal plasma were the lowest (P<0.05) in CSM, followed by 15% MOE, while total antioxidants activity was higher (P<0.05) for TEY+VC, CSM and 15% MOE than for TEY, being the highest for 15% MOE. Artificial insemination of estrous synchronized cows with semen extended by different types of extenders indicated that conception rate of cows was significantly (P<0.05) the highest for Friesian cows inseminated with post-thawed semen extended with CSM (87.5%), moderate with both TEY+VC and 15% MOE (75%) and the lowest with TEY(62.5%). Extender containing 15% MOE showed the cheapest cost (4.80 L.E./100 ml) in comparing with CSM (6.45 L.E./100 ml), TEY+VC (13.95 L.E./100 ml), and even the conventional TEY extender (13.85 L.E./100 ml). In conclusion, extender containing 15% *Moringa oleifera* leaf extract and 85% saline solution could be successfully used, as a source of antibiotics and antioxidants, in extenders of frozen bull semen, being with the cheapest cost as compared to tris-egg yolk or camel skim milk extenders.

Keywords: Bovine, semen, camel milk, Moringa oleifera, sperm function, enzyme activity.

INTRODUCTION

Artificial insemination (AI) in dairy cattle has attention as a replacement of natural service to improve the genetic potential of their livestock breeds by exploiting the germplasm of superior bulls (Anzar *et al*., 2003; Gordon, 2004). The latest assessment of AI worldwide quotes that around 252 million doses of frozen bovine semen and 11.6 million liquid doses of semen were produced in AI centers in 109 countries in 1998 (Thibier and Wagner, 2002).

There is still a need for cryopreservation process improvement, despite the intensive use of frozen semen in AI, because about 40-50% of the spermatozoa are damaged during freezing and thawing processes (Watson, 2000), resulting in the reduction of semen quality (Andrabi, 2009; Kumar *et al*., 2011). Cryo-damage during freeze-thawing of semen is due to high levels of polyunsaturated phospholipids sperm membrane (Andrabi, 2009). The lipid peroxidation (LPO) of unsaturated fatty acids in sperm membrane increases molecules of reactive oxygen species (ROS), which impairers mitochondrial system and dead/damage spermatozoa in semen (El-Sisy *et al*., 2007; Kadirvel *et al*., 2009). In mammalian semen, antioxidant potential is not enough to protect spermatozoa against oxidative stress during cryopreservation, whereas antioxidant level decreased during the freeze-thawing process by semen dilution and excessive generation of ROS molecules (Andrabi, 2009; Kumar *et al*., 2011). Therefore, the use of antioxidant in extender is recommended to reduce the cryo-damage to spermatozoa (Sansone *et al*., 2000; Andrabi, 2009). Natural antioxidants, such as vitamin C and E presented in semen of mammals against ROS as a protection of sperm cells from LPO and maintenance of cell membrane (Andrabi, 2009; Akhter *et al*., 2011). Therefore, they are used in extenders for improving the bovine sperm quality in frozen and liquid state (Andrabi *et al*., 2008; Akhter *et al*., 2011).

Medium composition used for semen dilution is an important factor influencing storage of frozen semen. Semen diluents are usually composed of a buffer medium to which cryoprotectants and other substances are added, for sperm protection during freeze–thawing (Sansone *et al*., 2000). Tris, milk and citrate-based diluents are also popular diluting media for storage of bovine semen (Kumar *et al*., 1992).

Moringa oleifera (MO) Lam (Syns. *Moringa* pterygosperm, family Moringaceae) is one of the most interesting trees, which is a native tree in Himalaya and currently spread almost world-wide (Bakhashwain *et al*., 2010; Sokunbi *et al*., 2015). The MO leaves are a good source of natural antioxidants to protect organism and cell from damage of oxidative stress (Rajanandh and Kavitha, 2010; Oparinde and Atiba, 2014) and also rich in essential amino acids (methionine, cystine, tryptophan, and lysine) and vitamins A, B and C with a high content of proteins (Ferreira *et al*., 2008; Mendieta-Araica *et al*., 2011). Extracts of MOL have been extensively studied as anti-tumour, anti-fertility, anti-fungal, anti-bacterial, and anti-oxidant agents (Sokunbi *et al*., 2015).

Extenders based on skim milk are used for semen dilution (Akhter *et al*., 2011). Supplementing milk as based semen extender with antioxidants did not result in superior semen quality, because milk casein that has antioxidant property, which reduces the requirement of the extra antioxidant supplementation (Foote *et al*., 2002). Fresh camel milk is rich in vitamin C, being three times of that in cow milk (Knoess, 1977), carotene (Kudabaer *et al*., 1972), and vitamin A (Anderson and Casals, 1973). Several authors used skim or whole camel milk as an extender for dilution of camel, buffalo and bovine semen (Zeidan *et al*., 2008; El-Nagar, 2008; El-Shennawy, 2013; Akhter *et al*., 2015).

Recently, MOE was used as a natural source of antioxidants and antibiotics in extender of bovine (Sokunbi *et al*., 2015), rabbit (Ghodaia, 2016) and sheep (El-Harairy *et*

al., 2016). Therefore, this study was designed to evaluate the possibility of using the optimal level of *Moringa oliefera* leaf aqueous extract (10, 15 or 20%) with 90, 85 or 80% saline water (MOLE) as a semen extender of bull semen in comparing with three extenders, including Tris-egg yolk (TEY) without or with vitamin C at a level of 0.9 mg/ml (TEY+VC), and camel skim milk (CSM).

MATERIALS AND METHODS

This study was conducted at Animal Production Research Station, El-Gemmezah, Gharbiya Governorate, belonging to Animal Production Research Institute (APRI), Agricultural Research Center, Egypt, during the period from February to May, 2017.

Animals:

Total of 5 sexually mature Friesian bulls (live body weight of 350-450 kg with 3-4 years of age) were used for collection of semen in the present study. Experimental bulls were with healthy appearance and free of diseases, being housed individually under semi-open sheds. Bulls were fed individually on daily diet containing concentrate fed mixture (CFM, 4 kg), fresh berseem (35 kg) and rice straw (6 kg). The CFM was composed of uncorticated cotton seed cake (25%), coarse wheat bran (44%), corn (15%), extracted rice bran (8.5%), molasses (3%), limestone (3%) and sodium chloride (1.5%). Drinking clean water was available all day time.

***Moringa oleifera* leaf extract (MOE):**

Moringa oleifera leaves were collected from the farm of El-Gemmezah Research Station, Egypt. The collected leaves was air-dried, powdered and kept for extraction. The resulting powder (300 g) was extracted in Pharmacology Department; Faculty of Pharmacy, Tanta University with one liter distilled water, left to stand for 72 hours at room temperature, and filtered with Whatman No. 1 filter paper. The crude aqueous extract was concentrated using rotary evaporator under reduced pressure at 45°C and kept at -20°C.

Semen collection:

Semen was collected twice a week from all bulls for 5 weeks (50 ejaculates from all bulls), using artificial vagina (IMV, France) at 40oC. Semen was collected before feeding at 7-8 a.m.. A bull was used as a teaser on day of semen collection. The collected ejaculates were taken immediately to the laboratory in water bath at 37 0C for evaluation and freezing processes only for ejaculates with mass motility of more than 70% on day of semen collection. On each collection day, all ejaculates were pooled and divided into 4 replicates for different treatments.

Experimental semen extenders:

Tris-extender:

Tris-egg yolk (TEY) extender with pH value of 6.8, and 280-300 mOsm/l contained tris (3.63 g), fructose (0.5 g), citric acid (1.99 g) streptomycin (100 mg) and penicillin (100.000 IU) per 100 ml distilled water, then 10 ml egg yolk and 7 ml glycerol were added to 83 ml Tris extender was used as a basal extender (TEY) without or with vitamin C at a level of 0.9 mg/ml (TEY+VC).

Camel skim milk extender (CSM):

Skim milk of pastoral camels was heated to 95°C for 10 min, then cooled to room temperature and filtrated for three times, and 10 ml egg yolk, 7 ml glycerol, penicillin and streptomycin were added to 83 ml milk to prepare 100 ml of CSM.

***Moringa oleifera* extract extenders:**

These extenders contained mainly saline solution (90, 85 and 80%) and different levels of MOE (10, 15 and 20%), beside fructose (0.5 g/100 ml extender), then 10 ml egg yolk and 7 ml glycerol were added to 83 ml of the extenders.

Type of experimental semen extenders:

Experiment I:

This experiment aimed to evaluate effect of extenders containing different levels of MOE as an antioxidants source with saline solution (SS), in comparing with conventional Tris-egg yolk (TEY), on sperm characteristics of Friesian bulls in post-diluted and post-equilibrated (for 4 h) semen. In this experiment four types of semen extenders were used, including, E1: TEY (control), E2: 10% MOE and 90% SS, E3: 15% MOE and 85% SS, and E4: 20% MOE and 80% SS. Semen was diluted at a rate of 1:10.

Experiment II:

This experiment aimed to evaluate the effect of the best results of the extender containing the optimum level of MOE (15% MOE, based on results of experiment I), CSM, and conventional TEY without supplementation or with 0.9 mg/ml vitamin C (TEY+VC), on sperm characteristics of Friesian bulls in post-diluted, post-equilibrated (for 4 h) and post-thawed semen. Total antioxidants and enzyme activity was determined in seminal plasma of post-thawed semen as well as fertility rate semen extended with different types of extenders was performed. The dilution was at a rate of 1:10.

Semen processing:

The diluted semen with each type of extenders was aspirated into medium-sized (0.25 ml) French straws, sealed with polyvinyl alcohol powder and equilibrated at 5^0C for 4 h. After equilibration, the straws were frozen in liquid nitrogen vapor, 5 cm above liquid nitrogen surface, for 10 min and then the straws were plunged into liquid nitrogen for storage. After storage for 4 weeks, frozen straws were thawed at 37^0C for 30 s in a water bath.

Semen evaluation:

Semen extended with each treatment level was evaluated in post-dilution, post-equilibrated and post-thawed for determining the percentages of individual motility (Amman and Hammerstedt, 1980), livability (Hackett and Macpherson, 1965), abnormality (Blom, 1983), acrosomal damage (Yanagimachi, 1982) and hypo-osmotic swelling test at 50 mOsm/l for 30 min, in term of curled tail percentage (El-Sherbieny, 2004). However, percentage of head to head agglutination was determined in post-thawed semen (Senger and Saacke, 1976). Frozen semen for at least one month was thawed at a rate of 37oC for 30s.

Total antioxidants and enzyme activity in seminal plasma:

After semen thawing, the seminal plasma was separated by centrifugation (4000 rpm, for 15 min) and stored at -20 °C until analysis. Concentration of total antioxidants (Koracevic *et al*., 2001), and activity of asprtate (AST) and alanine (ALT) aminotransaminases, and lactic dehydrogenase (LDH) in seminal plasma was measured using commercial kits (Salucea Netherlands) and spectrophotometer (JENWAY-6405UV/Vis) according to Young (1990).

Fertility trial:

About 48-72 h before artificial insemination, cows were i.m. injected with 3 ml Estrumate to induce estrus. Total of 32 cows synchronized with Estumate (PGF2α-

Essex Animal Helth Friesoythe, Germany) were artificially inseminated by semen of different types of extenders in experiment II (8 cows for each type). Animals were immediately inseminated with semen thawed at 37 ºC for 30 s using filled plastic AI gun close to the cervix. Pregnancy was diagnosed by rectal palpation on day 50-60 post-insemination. All inseminations were conducted by the same inseminator.

Statistical analysis:

Data were statistically analyzed by ANOVA using SPSS (2013). The significant differences among means were tested by Duncan multiple rang test (Duncan, 1955). The percentage values were subjected to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed values to percentages.

RESULTS AND DISCUSSION

Experiment I:

Sperm characteristics in post-diluted semen:

Sperm characteristics including percentages of motility, livability, abnormality, curled tail and acrosomal damage in post-diluted semen extended with E2 or E3 did not differ significantly from control semen extended with E1. However, semen extended with E4, resulted significantly in marked reduction in all characteristics as compared to E2, E3 and E1 (control, Table 1).

These results indicted save usage of SS supplemented with 10 or 15% MOE without antibiotics (streptomycin or penicillin) as observed for TEY extender with antibiotics in semen dilution. However, increasing level of MOE to 20% with 80% SS had negative effect on sperm characteristics.

Table 1. Effect of extender containing different levels of MOE on sperm characteristics in post-diluted bull semen.

Sperm characteristics (%)	Type of extender			
	E1 (TEY, control)	E2 (10% MOE)	E3 (15% MOE)	E4 (20% MOE)
Sperm motility	67.50±1.34^{a}	66.50±0.76^{a}	69.00±1.24^{a}	57.50±1.11^{b}
Sperm livability	66.90±1.32^{a}	65.70±1.46^{a}	68.00±1.10^{a}	56.60±1.21^{b}
Sperm abnormality	21.20±1.54^{a}	22.00±1.30^{a}	18.50±0.74^{a}	31.60±1.41^{b}
Acrosomal damage	21.00±1.33^{a}	21.90±1.08^{a}	18.70±0.93^{a}	30.20±1.47^{b}
Curled spermatozoa	67.10±0.97^{a}	66.00±1.09^{a}	68.20±0.71^{a}	56.90±0.97^{b}

Means denoted within the same row with different superscripts are significantly different at P<0.05.

Sperm characteristics in post-equilibrated semen:

In post-equilibrated semen, all sperm characteristics were significantly (P<0.05) the best with E3 and the poorest with E3 as compared to control (E1), while semen extended with E2 did not differ significantly from that in control (Table 4).

According to the results of sperm characteristics in post-diluted semen, also extender containing 15% MOE in 85% SS without antibiotics indicated beneficial effects on sperm function in post-equilibrated semen as compared to conventional TEY. Such results encouraged us to use this extender in freezing of bull semen in experiment II in comparing with TEY and other types of extenders.

Semen dilution in a suitable buffer is one of the important factors affecting sperm characteristics during cryopreservation. The success of semen cryopreservation depends on type and rate of semen dilution (Prasanna and Sreelatha, 2014). The beneficial effects of Tris-extender in dilution of Holstein semen was reported by Dandoush (2002). However, The observed maintenance of sperm characteristics in post-diluted semen extended with MOE at levels of 10 or 15% in comparing with TEY is in accordance with the recent results of some authors used MOE as a supplement to semen extenders. In this respect, mean progressive motility significantly increased in bull semen extended with glucose yolk citrate extender containing 12 ml of MO crude extract compared to 0, 8, and 16 ml MO crude extract (Sokunbi *et al*., 2015).

Table 2. Effect of extender containing different levels of MOE on sperm characteristics in post-equilibrated semen for 4 hours.

Sperm characteristics (%)	Post-equilibrated semen for 4 h			
	E1 (TEY, control)	E2 (10% MOE)	E3 (15% MOE)	E4 (20% MOE)
Sperm motility	61.50±1.06^{b}	60.50±1.38^{b}	68.00±0.81^{a}	51.00±1.00^{c}
Sperm livability	61.90±1.33^{b}	61.00±1.19^{b}	67.10±0.98^{a}	51.30±1.36^{c}
Sperm abnormality	27.20±1.39^{b}	28.40±1.08^{b}	19.40±0.97^{a}	36.70±1.75^{c}
Acrosomal damage	26.30±1.31^{b}	27.60±1.14^{b}	19.60±1.05^{a}	36.20±1.41^{c}
Curled spermatozoa	61.40±1.25^{b}	59.40±1.34^{b}	67.10±0.64^{a}	50.90±1.17^{c}

Means denoted within the same row with different superscripts are significantly different at P<0.05.

However, rabbit semen extended with tris-extender supplemented with 2 or 4 mg/l of MOE showed marked increase in sperm motility and longer preservation time at cool temperature (4-5 d) as compared to un-supplemented semen (Ghodaia, 2016). This suggests that MOE helps in maintaining intact sperm morphology and this could be attributed to the presence of tannins because of its astringent effect (Sokunbi *et al*., 2015). Moreover, Prasanna and Sreelatha (2014) stated that MOE treatment can act as effective modulators in reducing the toxicity in cells under oxidative stress that are capable of removing oxygen radicals and their products and/or repairing the damage caused by oxidation stress.

Generally, *Moringa oleifera* leaves (MOL) was reported to contain higher antioxidant contents such as β-carotene, vitamin C, and flavonoids (Amaglo *et al*. 2010; Gowrishankar *et al*., 2010), and polyphenols, tannins, anthocyanin, glycosides, and thiocarbamates (Luqman *et al*., 2012). Antioxidant and free radical scavenging activity of MOE (Chumark *et al*., 2008; Sreelatha and Padma, 2009) and anti-bacterial properties due to lipophilic compounds (Patel *et al*., 2011; Sokunbi *et al*., 2015) were reported. Improving mammalian cells exposed to variety of oxidative conditions by poly-phenolic compounds in MO leaves cells was reported by Srinivasan *et al*. (2007) and Devaraj *et al*. (2008) by enhancing the immune system scavenge of free radical and reduce the production of DNA mutations.

Experiment II:

In this experiment, the effect of extender containing 85% SS and 15% MOE (based on the best results of experiment I) in comparing with TEY, TEY plus 0.9 mg vitamin C/ml (TEY+VC) and CSM on sperm characteristics in post-diluted, post-equilibrated and post-thawed semen.

Sperm characteristics in post-diluted and post-equilibrated semen:

All sperm characteristics in post-diluted and post-equilibrated semen were significantly (P<0.05) higher in TEY+VC, CSM and 15% MOE than in TYE, being the highest in CSM, followed by 15% MOE and TEY+VC, respectively (Table 3).

Table 3. Effect of different types of extenders with antioxidant or MOE on sperm characteristics in post-diluted and post-equilibrated semen.

Sperm characteristics (%)	Post-diluted semen				Post-equilibrated (4 h) semen			
	TEY	TEY+VC	CSM	15% MOE	TEY	TEY+VC	CSM	15% MOE
Sperm motility	61.87^{b} ±2.30	70.00^{a} ±1.33	72.50^{a} ±2.11	71.87^{a} ±1.87	57.12^{b} ±1.59	66.50^{a} ±1.87	70.25^{a} ±1.81	67.75^{a} ±1.95
Sperm livability	62.25^{b} ±2.01	69.25^{a} ±1.43	70.75^{a} ±1.20	70.50^{a} ±1.08	57.12^{b} ±1.60	65.75^{a} ±1.20	69.62^{a} ±1.56	67.00^{a} ±1.28
Sperm abnormality	26.12^{b} ±1.32	18.25^{a} ±1.39	16.00^{a} ±1.21	17.00^{a} ±1.65	31.50^{c} ±1.89	22.75^{b} ±1.03	18.50^{a} ±1.22	21.25^{ab} ±1.23
Damage acrosome	26.37^{b} ±1.66	18.50^{a} ±0.86	16.12^{a} ±1.09	17.12^{a} ±1.39	31.12^{b} ±1.54	22.00^{a} ±1.26	18.12^{a} ±1.20	20.75^{a} ±1.44
Curled spermatozoa	59.50^{b} ±1.11	68.62^{a} ±1.25	70.87^{a} ±0.69	69.62^{a} ±1.14	55.75^{c} ±1.00	63.87^{b} ±1.38	68.37^{a} ±1.36	65.12^{ab} ±1.46

Means denoted within the same row with different superscripts are significantly different at P<0.05.
VC: Vitamin C was added at a level of 0.9 mg/ml.

Sperm characteristics in post-thawed semen:

It is worthy noting that all previous sperm characteristics, beside percentage of head to head agglutination achieved the same trend of change in post-thawed semen as recorded in post-diluted and post-equilibrated semen, being the highest in CSM, followed by 15% MOE and TEY+VC, respectively, and the lowest in TYE (Table 4).

Table 4. Effect of different types of extenders with antioxidant or MOE on sperm characteristics in post-thawed semen.

Sperm characteristics (%)	Type of extender			
	TEY	TEY+VC	CSM	15% MOE
Sperm motility	45.00±1.88^{c}	53.25±1.82^{b}	59.50±1.63^{a}	55.75±1.25^{ab}
Sperm livability	44.75±1.71^{b}	54.37±2.16^{a}	57.87±1.43^{a}	54.25±2.23^{a}
Sperm abnormality	41.50±2.17^{c}	31.00±0.98^{b}	24.75±1.43^{a}	29.62±0.98^{b}
Damage acrosome	40.25±2.25^{b}	32.25±2.01^{a}	27.00±1.28^{a}	31.25±2.23^{a}
Curled spermatozoa	43.00±1.28^{c}	55.75±1.83^{b}	61.00±1.10^{a}	57.37±2.08^{ab}
Head to head agglutination	42.25±0.79^{c}	50.12±1.14^{b}	57.50±1.89^{a}	51.87±0.81^{b}

Means denoted within the same row with different superscripts are significantly different at P<0.05.
VC: Vitamin C was added at a level of 0.9 mg/ml.

Good extender has been carefully monitored for osmolarity, free fatty acids, sulfhydryl groups, pH value and lipid oxidation as a part of quality control of the artificial breeding program thus (Pramanik and Raina, 1998). The observed improvement of post-diluted motility with CSM as compared to other extenders may be due to the appropriated osmolarity and pH value and increasing casein content and vitamin C (Kon, 1972; Knoess, 1977) in camel skim milk. Fresh camel milk has a high pH value, ranging between 6.5 and 6.7 (Shalash, 1980). Also, this phenomenon may be attributed to the better protection of lactose to spermatozoa against osmotic shock than other sugars or due to the mediate available energy and the osmotic balance of the extender (Zeidan *et al*., 2008). El-Nagar (2008) recommended that camel skim milk extender could be successfully used for cryopreservation of buffalo semen. Therefore, CSM extender may consider as a good extender for bull semen dilution.

In addition, the present results indicated similar positive effects of supplementing vitamin C in TEY extender or 15% MOE on improving sperm characteristics. Low toxicity and good water solubility of vitamin C has led to its use as an antioxidant additive and has been shown to have protective effects when added to an extender and this is in line with some previous studies (Ball *et al*., 2001; Arabi and Seidaie, 2008). These results may be explained based on the fact that vitamin C or MOE protects the spermatozoa by preventing from endogenous oxidative DNA and membrane damages. It is also believed that vitamin C works by scavenging superoxide anions and singlet oxygen and can protect the lipoproteins from detectable peroxidative damage; this finding suggested that ascorbic acid might be needed to protect sperm against reactive oxygen species (Asadpour *et al*., 2011).

Enzyme activity and total antioxidants activity in seminal plasma of post-thawed semen:

In seminal plasma of post-thawed semen, enzyme activities of AST, ALT and LDH were significantly (P<0.05) lower for CSM, 15% MOE and TEY+VC than in TEY, being the lowest in CSM, followed by 15% MOE. However, total antioxidants activity was significantly (P<0.05) higher in TEY+VC, CSM and 15% MOE than in TEY, being the highest for 15% MOE (Table 5).

These results are in association with sperm characteristics in post-thawed semen, in particular, acrosomal damage, HOS-t (curled spermatozoa) and head to head agglutination, indicated membrane integrity of spermatozoa in frozen semen extended with TEY supplemented with vitamin C, CSM and 15% MOE, being the best with CSM. However, addition of 15% MOE to 85% SS resulted in the highest activity of total antioxidants in seminal of post-thawed semen.

Such result indicated a negative relationship between activity of enzyme and total antioxidant in seminal plasma of bull frozen semen. Ghodaia (2016) reported that supplementation of rabbit semen with MOE increased total

antioxidant activity and decreased alkaline phosphatase (ALP) and LDH activities in seminal plasma of semen stored at 4-5°C. The obtained results of decreasing enzyme activity in seminal plasma of bull semen extended with CSM are similar to those obtained by El-Nagar (2008) in seminal plasma of buffaloes. In accordance with the results of MOE, Sofidiya *et al*. (2006) and Ogbunugafor *et al*. (2011) showed the effect of MOE as antioxidants, which are known to suppress ROS formation and free radicals. Extract of MO increased the production of antioxidants in the sperms (Abdou *et al*., 2012). Also, Luqman *et al*. (2012) found that MOE remove free radicals, activate antioxidant enzymes, and inhibit oxidases. Recently, Ghodaia (2016) reported that total antioxidant activity increased in blood of rabbit bucks orally treated with 60 mg (P<0.05) of MOE for 21 days.

Table 5. Effect of different types of extenders with antioxidant or MOE on activity of AST, ALT and LDH, and total antioxidants activity in seminal plasma of post-thawed semen.

Item	Type of extender			
	TEY	TEY+VC	CSM	15% MOE
AST (IU/l)	40.25±0.94^{a}	24.37±0.73^{b}	22.50±0.60^{b}	23.87±0.55^{b}
ALT (IU/l)	28.50±0.87^{a}	22.00±0.63^{b}	18.75±0.53^{c}	21.00±0.53^{b}
LDH (IU/l)	317.75±1.60^{a}	264.75±1.97^{b}	238.62±1.15^{d}	246.87±1.85^{c}
Total antioxidants (mmol/l)	2.37±0.32^{b}	3.87±0.48^{a}	4.25±0.25^{a}	4.87±0.35^{a}

Means denoted within the same row with different superscripts are significantly different at P<0.05.
VC: Vitamin C was added at a level of 0.9 mg/ml.

Fertility rate:

Artificial insemination of estrous synchronized cows with semen extended by different types of extenders indicated that conception rate of cows was significantly (P<0.05) the highest for Friesian cows inseminated with post-thawed semen extended with CSM (7/8, 87.5%), moderate with both TEY+VC and 15% MOE (6/8, 75%) and the lowest with TEY (5/8, 62.5%, Fig. 1). Generally, these findings indicated association between conception rate and improving sperm characteristics and enzyme activity in post-thawed bull semen. Similar results were obtained by El-Nagar (2008), who recorded higher conception rate of buffalo cows inseminated with semen extended CSM with or without antioxidant supplementation as compared to other types of extenders.

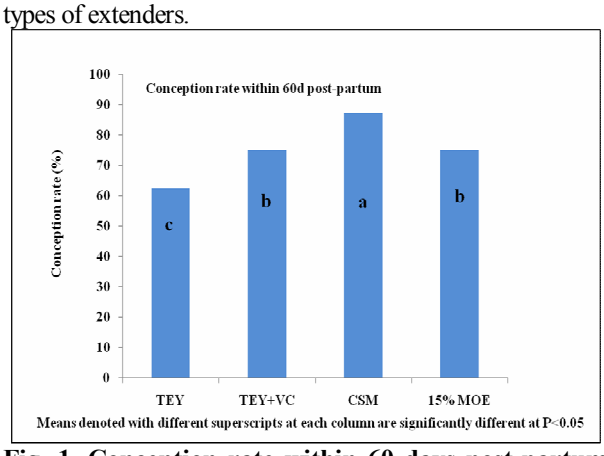

Fig. 1. Conception rate within 60 days post-partum of Friesian cows inseminated with different types of extenders.

Economic efficiency of different types of extenders:

It is worthy to noting that, beside the beneficial effects of 15% MOE extender in bovine frozen semen, this extender showed the cheapest cost (4.80 L.E./100 ml) in comparing with CSM (6.45 L.E./100 ml), TEY+VC (13.95 L.E./100 ml), and even the conventional TEY extender (13.85 L.E./100 ml).

CONCLUSION

In conclusion, extender containing 15% Moringa oleifera leaf extract and 85% saline solution could be successfully used, as a source of antibiotics and antioxidants, in extenders of frozen bull semen, being with the cheapest cost as compared to tris-egg yolk or camel skim milk extenders, but the extender containing 15% moringa oleifera leaf extract showed the cheapest cost.

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تأثير بعض مخففات السائل المنوي كمصدر طبيعي لمضادات الأكسدة على جودة السائل المنوي المجمد لطلائق الفريزيان

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تم تصميم هذة التجربة لتقييم إمكانية استخدام المستوى الأمثل من المستخلص المائي لأوراق المورنجا (10 , 15 و 20٪) مع المحلول الملحي (90 , 85 و80٪) كمخفف للسائل المنوي لطلائق الفريزيان مقارنة بثلاثة مخففات هي مخفف الترس-صفار البيض مع أوبدون إضافة فيتامين ج بمستوى 0.9 ملجرام/مل ومخفف لبن الجمال الفرز. تم تجميع السائل المنوي من خمس طلائق فريزيان (350-450 كجم وزن حي وعمرها 3-4 سنوات) مرتين أسبوعيا لمدة خمس أسابيع باستخدام المهبل الاصطناعي. وتم اختيار القذفات ذات الحركة الجماعية أكبر من أو يساوي 70٪ وتم خلطها وتقييمها وقسمت إلى أربعة مكررات وتم تجميدها. تم تخفيف السائل المنوي في مخفف الترس-صفار البيض بدون اضافه (المعاملة الأولى) أومع إضافة فيتامين ج بمعدل 0.9 ملجرام/مل (المعاملة الثانية) ولبن الجمال الفرز (المعاملة الثالثة) والمستخلص المائي لأوراق المورنجا (15٪) مع 85٪ محلول ملحي (المعاملة الرابعة , بناءا على أفضل نتائج مستويات المستخلص المائي لأوراق المورنجا) وتم عمل فترة موازنة على درجة حرارة 5°م لمدة 4 ساعات ثم التجميد في النيتروجين السائل. بعد الحفظ لمدة 4 أسابيع تم اسالة قصيبات السائل المنوي المجمدة على درجة حرارة 37°م لمدة 30 ثانية , وتم تقييم السائل المنوي بعد التخفيف وبعد الموازنة وبعد الاسالة وتم قياس خصائص السائل المنوي متضمنة النسبة المئوية لكل من الحركة الفردية والحيوانات المنوية الحية والشاذة وذات الأكروسوم الغير سليم والملتوية الذيل بالأضافة الى قياس نسبة التقابل للرأس بالرأس في السائل المنوي بعد الاسالة فقط , تم قياس النشاط الأنزيمي لكل من (AST , ALT و LDH) ونشاط مضادات الأكسدة الكلي في بلازما السائل المنوي بعد الاسالة. تم حساب معدل الخصوبة للأبقار الملقحة بأنواع المخففات المستخدمة. أظهرت النتائج ان: 1-النسبة المئوية للحركة الفردية والحيوانات المنوية الحية والشاذة وذات الأكروسوم الغير سليم والملتوية الذيل بعد التخفيف والموازنه والتجميد بالأضافه الى نسبة التقابل للرأس بالرأس في بعد الاسالة فقط كانت الأعلى معنويا في مخفف لبن الجمال الفرز يليه مخفف المستخلص المائي لأوراق المورنجا (15٪) ثم مخفف الترس مضافا إليه فيتامين ج وأقلها فى مخفف الترس العادى. 2- كان النشاط الإنزيمى لكل من AST،ALT وLDH في بلازما السائل المنوي بعد الأساله الأقل معنويا في مخفف لبن الجمال الفرز، يليه مخفف المستخلص المائي لأوراق المورنجا (15٪). 3- زاد النشاط الكلى لمضادات الأكسدة في بلازما السائل المنوي بعد الأساله معنويا فى مخففات الترس مع فيتامين ج ، لبن الجمال الفرز والمستخلص المائي لأوراق المورنجا (15٪) مقارن بمخفف الترس العادى وكان أعلاها فى المستخلص المائي لأوراق المورنجا (15٪).4- كان معدل الحمل للأبقار الملقحة بالسائل المنوي المخفف بلبن الجمال الفرز بعد الاسالة الأعلى معنويا (87.5٪) وكان متوسط في مخففى الترس مع فيتامين ج و 15٪ مورنجا (75٪) و الأقل في مخفف الترس العادى (62.5٪).5- أظهر مخفف المستخلص المائي لأوراق المورنجا (15٪) أقل تكلفة (4.80 جنيها مصريا لكل 100 مل مخفف) مقارنة بمخفف لبن الجمال الفرز (6.45 جنيها مصريا لكل 100 مل مخفف) و الترس مضافا اليه فيتامين ج (13.95 جنيها مصريا لكل 100 مل مخفف) وحتى مخفف الترس العادي (13.85 جنيها مصريا لكل 100 مل مخفف). الخلاصة: المخفف المحتوي على المستخلص المائي لأوراق المورنجا (15٪) مع محلول ملحي (85٪) يمكن أن يستخدم بنجاح كمخفف للسائل المنوي المجمد لطلائق الفريزيان كمصدر للمضادات الحيوية ومضادات الأكسدة وكان الأرخص في التكلفة مقارنة بمخفف لبن الجمال الفرز أو الترس – صفار البيض.