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Effect of Addition of *Moringa Oleifera* Extract to Tris Extender on the Preservability of Cattle Bull Semen

Reda I El-Sheshtawy and Walid S El-Nattat*

Animal Reproduction and Artificial Insemination Dept., National Research Centre, Dokki, Giza – Egypt
***Corresponding author:** elnattat@gmail.com

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ABSTRACT

Moringa oleifera extract is a strong natural antioxidant that when was added to the semen extenders, it induced a cryoprotection to spermatozoa effect through elimination of the excess free radicals. So, the existing study intended for clarification of the consequence of extract of *Moringa* leaves (MLE) on bull spermatozoa after chilling and cryopreservation. MLE concentrations were 0% (control), 10, 20, 30, 40 and 50% (v/v) [MLE: TCF (Tris-citric-fructose diluent)] then 20% egg yolk was added, then the extended semen was assigned to the freezing protocol. Then, it was evaluated for (motility, alive, abnormality %, sperm membrane integrity % before and after freezing). Sperm motility was kept high with the concentration 10, 30 and 40% of MEEY till 8 days of chilling. The concentration 20% maintained sperm motility high till 7 days of chilling. Addition of MLE to TCF significantly ($P<0.002$) improved sperm motility in all concentrations except the 50% moringa enriched extender with egg yolk (MEEY) where sperm motility was maintained as the control. The use of MEEY maintained % of alive sperms and % of normal spermatozoal membrane (HOST%) as good as the control. In conclusion: moringa as a herbal supplement to semen diluents enhanced preservation in cooled and cryopreserved cattle bull semen.

Key words: Cattle; Moringa extract, semen, Cryopreservation.

INTRODUCTION

Bull semen freezing frequently exert a hazardous cause for the oxidative stress on sperm due to decreased levels of antioxidant enzymes and the spermatozoal membrane become more liable to oxidative damage (El-Sisy *et al*., 2007; Belhan and Gülyüz, 2018) which affect the membrane integrity (Awda *et al.*, 2009). Phytochemicals as antioxidants have a strong preservative effect for cellular viability and metabolic function of frozen bovine spermatozoa (Camara *et al*., 2011). Recently, the phyto-products have gained interest worldwide upon using as supplements. They have been used as herbal supplement which enhance the healthy status. Moringa is of these herbs that possesses high nutritive benefits and includes many metabolic active substances including amino acids, minerals, vitamins, phenolic compounds, flavonoids and others. Recently, natural product has acquired importance in different countries of the world. Many of the natural products were manufactured into herbal enrichment which are specified to improve the healthy condition. *Moringa oleifera* Lam is an example of the natural products which has variable medicinal values. *Moringa oleifera* is an extremely nutritive herb distributed in different countries of the world. It has multiple medicinal applications with different nutritional functions. Different pieces of this plant are rich in minerals, protein, vitamins, essential amino acids and phenolics (Mehta *et al*., 2011). Moringa has strong antioxidant property through elevation of the level of glutathione enzyme (Fakurazi *et al.*, 2008b). Ghasi *et al*. (2000) stated the hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* and considered moringa a potential life saver (incredible leaves). Moringa supplemented hens had higher laying egg rate and high egg mass produced daily (Mohammed *et al*., 2012). Moringa increased libido, sperm count, production and motility by increasing testosterone level through inhibiting 6 beta-hydroxylation of testosterone (Fatoba *et al.*, 2013).

No available literatures were found for discussing the beneficial properties for using the moringa extract in preserving diluted bull semen, so the target of this study was to clarify the impact of moringa oleifera extract on semen preservability in cattle.

MATERIALS AND METHODS

The different semen extenders

TRIS extender: The Tris-citric-fructose extender (TCF) was set as recorded by Foote, Brockett and Kaproth (2002). While the addition of 20% whole egg yolk (TCFY).

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Moringa enriched extender [MEE]: dried *Moringa oleifera* leaves extract (MLE) was prepared via well pulverizing of dried leaves in a blender. This powder was soaked in tris base diluent (2.5g/45 ml) (Fakurazi *et al.*, 2008a, b) and then stored in refrigerator (10°C) for five days with daily stirring, the whole mixture was filtered through a gauze and finally centrifuged to get the supernatant of cold MLE. 6 tubes (one TCFY and 5tubes of MEE with 20% whole egg yolk, MEEY). MEE concentrations were 0/5.0 ml (control), 0.5/4.5 ml (10%), 1/4 ml (20%), 1.5/3.5 ml (30%), 2/3 ml (40%), 2.5/2.5 ml (50%) (v/v) [MLE: TCF] then 20% egg yolk was added, mixed and finally stored at -20°C.

Semen Collection and Initial assessment

Three cattle bulls (aged 3.5-5 years) at the Semen Freezing Center, Ministry of Agriculture, were kept for semen collection. The bulls were in good general health conditions (600-800 Kg body weight), free from general and genital diseases and kept under standard nutrition and good management. Semen was harvested using an artificial vagina every week for 18 weeks. Semen aliquots were primarily assessed for spermatozoal motility and concentration. Semen samples having minimum sperm motility (70%) and morphological sperm abnormalities were pooled to get enough semen volume and to avoid the individual bull variation. Samples of semen were hold at 37°C in the water bath before extension. The experimental design was approved and certified by the National Research Centre Medical Research Ethics Committee (Egypt) with an ethical certificate no. 17157.

Semen freezing protocols

Semen aliquots were extended in TCF diluent and considered as a control and other collected semen specimens were extended in TCF diluents enclosing the diverse concentrations of moringa extract to offer 60 million sperm/ml. Diluted semen was cooled slowly for 2 hrs to 5°C and equilibrated for 2 hrs. Semen was filled in 0.25 ml polyvinyl French straws. After equilibrium interval, the straws were put horizontally on a special rack and freezed 4 cm above liquid nitrogen vapor for 10 minutes and then plunged in liquid nitrogen. A portion of diluted semen from control and each concentration of the additives were chilled at 5°C for 10 days and sperm motility was assessed every day.

Evaluation of Semen characteristics

The evaluation was implemented on post-thawed bull spermatozoa. Also, sperm motility was evaluated for raw semen, 2 hours after cooling and in chilled semen every day up to 10 days. Frozen straws were thawed at 37°C/1 minute. The semen parameters (motility, alive, morphological abnormality and sperm membrane status (HOST) %) were done (Salisbury *et al*., 1978).

Statistical analysis

Data were analyzed by means of the SPSS (2005) computerized program v. 14.0 to calculate the analysis of variance (ANOVA) for the different criteria of the control and different supplement samples. Significant difference between means was calculated using Duncan test at $P<0.05$.

RESULTS

Impact of *Moringa oleifera* supplemented extender on bull sperm motility during chilling

Sperm motility was kept high with the concentration 10, 30 and 40% of MEEY till 8 days of chilling (46.67±1.67, 45.00±5.00 and 41.67±4.41). The concentration 20% maintained sperm motility high till 7 days of chilling (55.00±7.64) [Table 1].

Impact of *Moringa oleifera* supplemented extender on bull post - thawing sperm parameters

Supplementation TCF with MLE significantly ($P<0.002$) improved sperm motility in all concentrations except the 50% MEEY where sperm motility was maintained as the control Table 2. The use of MEEY maintained % of alive sperms and % of normal sperm membrane (HOST%) as good as the control.

DISCUSSION

Sperm Cryopreservation is of an extreme interest (Medeiros *et al*., 2002). According to Gadea *et al*. (2008), Uysal and Bucak (2007); Bucak *et al.* (2008), decreasing the sperm stresses after cooling, freezing and thawing and thereby enhancing sperm livability and potentiality of fertilization was attained by adding cryopreservatives in the semen diluent (Gadea *et al*., 2008; Uysal and Bucak, 2007; Bucak *et al*., 2008). Cryopreservation caused chemical, physical, and mechanical injures to sperm membranes (Watson, 2000), which were related to temperature changes, over accumulation of reactive oxygen species (ROS), conversions in the transition from the lipid phase, and osmotic stress (Câmara *et al.*, 2011; Ortega *et al*., 2009). Also, the extra release of ROS resulted in oxidative damage that included morphological changes of the spermatozoal membranes, decrease of intracellular ATP levels in the sperm cells with consequent lowered motility and livability of frozen spermatozoa (Baumber *et al.*, 2000; Agarwal *et al.*, 2005). Seminal plasma has strong source of free radicals' scavengers, thus protecting spermatozoa, involving catalase superoxide dismutase, glutathione peroxidase, and antioxidants of small molecular weight such as ascorbic acid and α-tocopherol (Aitken and Baker, 2004; Sikka, 2004). The herbal additives antioxidants minimized the hazardous effect of the oxygen free radicals. Nowadays, there is a great worldwide interest with the beneficial synergistic properties of herbal supplements and their multiple compounds as compared to the single purified active fractions (Seeram *et al*., 2004). Semen freezing caused cryoinjury to spermatozoa leading to reduction in semen quality (Watson, 2000), but it is important to preserve the supergenetic constitution of our local breeds of cattle. Semen freezing was associated with cryo-damage caused by overproduction of oxygen free radicals (Agrawal *et al*., 2005), so, the natural supplement to the extender enhanced the antioxidant effect and consequently improved the fertilizing capability of frozen spermatozoa (Gadea *et al*., 2008). The results of the current trials revealed an improving in sperm motility using the concentrations 10, 30 and 40% of MEEY till 8 days of chilling cattle semen.

Table 1: Impact of *Moringa oleifera* enriched extender on bull sperm motility during chilling.

Time	2 Hours	Days								
Treatment		1	2	3	4	5	6	7	8	9
Control	90.00^{a}	88.33^{a}	88.33^{a}	75.00^{a}	73.33^{a}	68.33^{a}	65.00^{a}	58.33^{a}	51.67^{a}	36.67^{a}
	± 0.00	± 1.67	± 1.67	± 5.00	± 6.01	± 6.01	± 2.89	± 3.33	± 1.67	± 1.67
10% MEEY	90.00^{a}	90.00^{a}	90.00^{a}	83.33^{a}	80.00^{a}	76.67^{a}	70.00^{a}	56.67^{a}	46.67^{ab}	33.33^{a}
	± 0.00	± 0.00	± 0.00	± 3.33	± 5.00	± 3.33	± 5.00	± 3.33	± 1.67	± 1.67
20% MEEY	90.00^{a}	90.00^{a}	90.00^{a}	83.33^{a}	78.33^{a}	75.00^{a}	73.33^{a}	55.00^{a}	38.33^{bc}	25.00^{a}
	± 0.00	± 0.00	± 0.00	± 3.33	± 4.41	± 5.00	± 4.41	± 7.64	± 4.41	± 5.00
30% MEEY	90.00^{a}	90.00^{a}	90.00^{a}	83.33^{a}	76.67^{a}	71.67^{a}	66.67^{a}	56.67^{a}	45.00^{ab}	31.67^{a}
	± 0.00	± 0.00	± 0.00	± 3.33	± 6.67	± 4.41	± 3.33	± 4.41	± 5.00	± 4.41
40% MEEY	90.00^{a}	85.00^{ab}	85.00^{ab}	78.33^{a}	75.00^{a}	73.33^{a}	66.67^{a}	56.67^{a}	41.67^{ab}	26.67^{a}
	± 0.00	± 2.89	± 2.89	± 7.26	± 7.64	± 6.67	± 6.01	± 4.41	± 4.41	± 4.41
50% MEEY	90.00^{a}	81.67^{b}	81.67^{b}	70.00^{a}	66.67^{a}	61.67^{a}	60.00^{a}	45.00^{a}	30.00^{c}	18.33^{a}
	± 0.00	± 1.67	± 1.67	± 5.00	± 6.67	± 4.41	± 5.00	± 5.00	± 2.89	± 4.41
F-value	----	5.16	5.16	1.36	0.59	1.14	0.98	0.99	4.34	2.96
P-value		0.0093	0.0093	0.3073	0.7114	0.3904	0.4674	0.4641	0.0173	0.0575

Different letter superscripts (a, b…) indicate a significant difference between means within column using the multiple range Duncan's test at P<0.05.

Table 2: Impact of *Moringa oleifera* enriched extender on bull post- thawing sperm parameters

Parameters treatment	Motility %	Alive %	Abnormal %	HOST %
Control	40.83±5.54^{c}	80.50±3.75^{a}	16.80±1.62^{b}	80.50±2.02^{a}
10% MEEY	56.67±2.47^{a}	86.90±3.41^{a}	16.55±2.63^{b}	80.50±1.44^{a}
20% MEEY	57.50±2.81^{a}	85.85±3.95^{a}	25.20±0.69^{a}	75.50±2.02^{a}
30% MEEY	55.00±2.24^{ab}	81.10±4.68^{a}	20.30±2.48^{ab}	81.00±1.73^{a}
40% MEEY	46.67±2.47^{bc}	85.60±2.66^{a}	21.25±2.45^{ab}	78.00±2.31^{a}
50% MEEY	44.17±2.01^{c}	81.70±4.45^{a}	17.10±0.64^{b}	82.00±2.89^{a}
F-value	5.11	0.53	3.08	1.28
P<	0.0017	0.7529	0.0513	0.3345

Different letter superscripts (a, b…) indicate a significant difference between means within column using the multiple range Duncan's test at P<0.05.

This indicated its applicability in AI till 8 days while the concentration 20% maintained sperm motility high till 7 days post chilling. The findings of the existing investigation exhibited improved post thawing sperm motility in all MEEY except the concentration of 50%. Also, all concentrations of moringa extract added maintained the % of alive sperm and % of normal sperm membrane (HOST%) of frozen semen as good as the control. These results may be related to the antioxidant potency of *Moringa oleifera* extract at these concentrations through increasing the antioxidant effect of catalase, glutathione and superoxide dismutase and with reduced sperm membrane phospholipids peroxidation (Moyo *et al*., 2012).

Conclusions

Moringa as a herbal supplement to semen diluents enhanced preservation in cooled and cryopreserved cattle bull semen. In all concentrations, Sperm motility was kept during chilling up to 7-8 days and also in post-thawing except with the concentration 50%.

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Author contribution: both authors had shared the preparation of the moringa extraction and semen extender, the semen collection, dilution and freezing protocol, evaluation of semen and the preparation of the present manuscript for publication.

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