



Assessment of Buffalo Semen Preservability Using Tris Extender Enriched With *Moringa Oleifera* Extract

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THE aim of the present study was to explore the effect of *Moringa oleifera* leaves extract (MLE) on buffalo spermatozoa during cooling and cryopreservation. Pooled bull semen were diluted by Tris-Citrate-Fructose (TCF) diluent supplemented with MLE and allocated in 6 concentrations which were 0% control, 10, 20, 30, 40, 50% (v/v) [MLE: TCF] then 20% egg yolk was added, mixed and finally stored at -20°C until used. Diluted semen was cooled slowly up to 5 °C and equilibrated for 4 h. Semen was packed into 0.25 mL polyvinyl straws. After equilibration periods, the straws were exposed to liquid nitrogen (LN₂) vapor for 10 minutes and were then dipped in LN₂. Extended semen was subjected to evaluation (motility, alive %, abnormality %, and intact sperm membrane (HOST) %) in both chilled and frozen semen. Sperm motility was significantly (P<0.0001) kept high after 11 days of chilling with the concentration 10 and 20%, also it was kept significantly (P<0.0001) high with the concentration 30 and 40% up to 10 days. The use of MEEY had significantly (P<0.0001) improved sperm motility% with the concentration 10 and 20% compared to the control, while the other concentrations didn't exhibit any effect. On the other hand, MEEY had maintained alive sperm, intact spermatozoa membrane% and abnormal sperm % as good as the control with all the concentrations of MEE used in the present study. Then, it could be concluded that 10-20% of *Moringa oleifera* leaves extract (under the contemporary conditions of extraction in this study) as a natural additive to semen extenders improved preservability in both chilled and frozen bull semen.

Keywords: Buffalo, Semen, Preservation, Moringa.

Introduction

There are multiple advantages upon application of semen freezing in the breeding programs. The superior benefit is the durable storage of genetic material.

Freezing of buffalo semen is considered an essential biotechnological application also, it is accompanied with over production of reactive oxygen species that cause oxidative cryoinjury to spermatozoa, thus lowering its vitality [1] and consequently affecting viability and its fertilizing capacity [2,3,4]. Liability to lipid peroxidation differs according to freezing protocol, the extender composition and the antioxidant concentration

[5]. Addition of antioxidant to the extender exerts advantageous impact on spermatozoa via reducing the cryoinjury induced by oxygen free radicals [6, 7]. Recently, the use of herbal natural product has gained interest among the world population and their extracts are used as natural additives in semen extenders. *Moringa oleifera* is a superior quality plant with valuable nutritional property rich in phenolics, vitamins and minerals [8]. The nutritive ingredients of *Moringa oleifera* induce a synergistic antioxidant effect [9], thus minimizing the oxidative stress to spermatozoa during the freezing process. The dietary supplementation of *Moringa oleifera* ameliorates libido, sperm concentration and forward motility [10]. No

available literatures are presented illustrating the benefits of using *Moringa oleifera* extract as an additive to preserve extended semen in bovines, so the current investigation aimed to clarify the effect of *Moringa oleifera* on semen preservability on buffalo semen.

Materials and Methods

Preparation of variable semen extenders:

TRIS base extender: Tris-citric acid-fructose diluent (TCF) was set as recorded by Foote et al. [11]. 20% whole egg yolk (TCFY) was added.

Moringa enriched extender [MEE]: Dried *Moringa oleifera* leaves extract (MLE) was prepared via well grinding of dried leaves in a blender. This powder was soaked in Tris base extender (2.5 g / 45 ml, for one hour) [9, 12] and kept at (10 °C) for five days with daily stirring, the whole mixture was filtered through a gauze and finally centrifuged to get the supernatant of MLE. six tubes (one TCFY and 5 tubes of MEE with 20% whole egg yolk, MEEY). MEE concentrations (in ml) / Tris basic extender (in ml) were 0/5.0 (control, 0%), 0.5/4.5 (10%), 1/4 (20%), 1.5/3.5 (30%), 2/3 (40%), 2.5/2.5 (50%) (v/v) [MLE: TCF] then 20% egg yolk was added, mixed and finally kept at -20°C.

Semen Collection and Initial Evaluation

Semen from three mature buffalo bulls kept at Semen Freezing Center, General Organization for Veterinary Services Ministry of Agriculture, Abbasia, Egypt, were used. Ejaculates were collected by means of artificial vagina at weekly intervals for 18 weeks. Semen samples were primarily assessed for sperm motility and sperm concentration. Ejaculates fulfilling minimum sperm motility (70%) and normal sperm morphology were pooled in order to have sufficient semen for a replicate and to exclude the bull effect. Semen was held for 10 minute at 37°C in the water bath before dilution.

Semen processing

Semen samples were extended with TCF extender and used as control and other aliquots of pooled semen samples were extended with TCF extenders containing the variable concentrations of moringa extract to reach concentration of 60 million sperm/ml. Diluted semen was cooled slowly (approximately for 2 hrs) to 5°C and equilibrated for 2 hrs. Semen was filled into 0.25

ml polyvinyl French straws. After this period, the straws were placed horizontally on a rack and frozen in vapor 4 cm above liquid nitrogen for 10 minutes and were then dipped in liquid nitrogen. A portion of diluted semen from control and each concentration of the extract were kept at 5 °C for 7-10 days (chilling) with daily evaluation of sperm motility.

Evaluation of Semen Quality Parameters

The assessment was implemented on freeze-thawed bull spermatozoa. Also, sperm motility was evaluated for raw semen, 2 hours after cooling and chilled semen daily up to 7-10 days. Frozen straws were thawed at 37°C / 1 minute. The semen characteristics studied were (motility, alive, abnormality and hypoosmotic swelling test (HOST) %) [13].

Statistical analysis

Statistical analysis data were computed using the SPSS [14] computerized program v. 14.0 to calculate the analysis of variance (ANOVA) for the different parameters between control and additives replications. Significant difference between means was calculated using Duncan multiple range test at $P < 0.05$.

Results

Effect of *Moringa oleifera* enriched extender on buffalo sperm motility during chilling.

Sperm motility was significantly ($P < 0.0001$) kept high after 11 days of chilling with the concentration 10 and 20% (51.25 ± 1.25 and $42.50 \pm 1.44\%$, respectively) as compared to the control, also significantly ($P < 0.0001$) kept high with the concentration 30 and 40% (42.50 ± 1.44 and 43.75 ± 2.39 , respectively) up to 10 days if compared to the control [Table 1].

Effect of *Moringa oleifera* enriched extender on buffalo post thawing sperm characteristics.

The use of MEEY had significantly ($P < 0.0001$) improved sperm motility% with the concentration 10 and 20% (47.50 ± 2.81 and 48.33 ± 3.33 , respectively) as compared to the control, while the other concentrations didn't explore any effect [Table 2].

Additionally, MEEY had maintained alive sperm, intact spermatozoa membrane% and abnormal sperm % as good as the control with all the concentrations of MEE used in the current study.

TABLE 1. Effect of *Moringa oleifera* enriched extender on buffalo sperm motility during chilling

| Time Treatment | 2 Hours | Days | | | | | | | | | | |
|----------------|--------------------|--------------------|--------------------|--------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|--------------------|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| Control | 88.75 ^a | 72.50 ^a | 70.00 ^a | 61.25 ^b | 52.50 ^c | 43.75 ^d | 32.50 ^d | 30.00 ^d | 26.25 ^d | 26.25 ^c | 17.50 ^c | 15.00 ^d |
| | ± 1.25 | ± 9.24 | ± 8.90 | ± 5.91 | ± 4.79 | ± 3.75 | ± 3.23 | ± 2.89 | ± 2.39 | ± 2.39 | ± 1.44 | ± 2.04 |
| 10% MEE | 90.00 ^a | 78.75 ^a | 86.25 ^a | 80.00 ^a | 80.00 ^a | 77.50 ^a | 73.75 ^a | 71.25 ^a | 67.50 ^a | 62.50 ^a | 56.25 ^a | 51.25 ^a |
| | ± 0.00 | ± 9.66 | ± 1.25 | ± 0.00 | ± 0.00 | ± 1.44 | ± 1.25 | ± 2.39 | ± 1.44 | ± 2.50 | ± 2.39 | ± 1.25 |
| 20% MEE | 90.00 ^a | 86.25 ^a | 86.25 ^a | 81.25 ^a | 76.25 ^a | 75.00 ^{ab} | 70.00 ^{ab} | 68.75 ^{ab} | 66.25 ^{ab} | 58.75 ^{ab} | 51.25 ^{ab} | 42.50 ^b |
| | ± 0.00 | ± 2.39 | ± 2.39 | ± 1.25 | ± 2.39 | ± 2.04 | ± 2.04 | ± 3.15 | ± 3.15 | ± 1.25 | ± 3.15 | ± 1.44 |
| 30% MEE | 91.25 ^a | 87.50 ^a | 86.25 ^a | 80.00 ^a | 77.50 ^a | 73.75 ^{ab} | 72.50 ^a | 66.25 ^{ab} | 62.50 ^{ab} | 53.75 ^{ab} | 42.50 ^b | 35.00 ^c |
| | ± 1.25 | ± 2.50 | ± 3.15 | ± 0.00 | ± 1.44 | ± 1.25 | ± 1.44 | ± 2.39 | ± 3.23 | ± 2.39 | ± 1.44 | ± 2.04 |
| 40% MEE | 88.75 ^a | 86.25 ^a | 85.00 ^a | 76.25 ^a | 73.75 ^{ab} | 67.50 ^{bc} | 63.75 ^b | 61.25 ^b | 57.50 ^b | 51.25 ^b | 43.75 ^b | 36.25 ^c |
| | ± 1.25 | ± 2.39 | ± 2.04 | ± 3.75 | ± 3.15 | ± 4.33 | ± 3.15 | ± 4.27 | ± 3.23 | ± 2.39 | ± 2.39 | ± 2.39 |
| 50% MEE | 88.75 ^a | 85.00 ^a | 78.75 ^a | 72.50 ^a | 66.25 ^b | 60.00 ^c | 56.25 ^c | 48.75 ^c | 42.50 ^c | 27.50 ^c | 12.50 ^c | 2.50 ^c |
| | ± 1.25 | ± 2.89 | ± 1.25 | ± 3.23 | ± 2.39 | ± 3.54 | ± 2.39 | ± 3.15 | ± 3.23 | ± 5.95 | ± 6.29 | ± 2.50 |
| F-value | 1.00 | 1.02 | 2.56 | 5.63 | 13.54 | 18.21 | 43.14 | 25.71 | 32.32 | 24.87 | 30.03 | 82.75 |
| P< | 0.4457 | 0.4375 | 0.0644 | 0.0027 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |

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

TABLE 2. Effect of *Moringa oleifera* enriched extender on buffalo post thawing sperm characteristics

| Parameters Treatment | Motility % | Alive % | Abnormal % | HOST % |
|----------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Control | 31.67 ^b ± 1.67 | 76.00 ^a ± 2.31 | 21.33 ^a ± 3.48 | 76.00 ^a ± 1.15 |
| 10% MEE | 47.50 ^a ± 2.81 | 82.67 ^a ± 1.76 | 22.00 ^a ± 0.00 | 75.00 ^a ± 3.71 |
| 20% MEE | 48.33 ^a ± 3.33 | 84.00 ^a ± 2.00 | 22.67 ^a ± 1.76 | 76.67 ^a ± 3.71 |
| 30% MEE | 29.17 ^b ± 3.52 | 76.00 ^a ± 3.21 | 21.33 ^a ± 2.40 | 78.00 ^a ± 5.03 |
| 40% MEE | 17.50 ^c ± 4.03 | 79.33 ^a ± 3.48 | 19.33 ^a ± 1.33 | 79.33 ^a ± 0.67 |
| 50% MEE | 15.83 ^c ± 5.39 | 77.33 ^a ± 2.33 | 19.67 ^a ± 2.33 | 81.33 ^a ± 2.40 |
| F-value | 14.90 | 1.76 | 0.36 | 0.62 |
| P< | 0.0001 | 0.1965 | 0.8642 | 0.6903 |

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

Discussion

Recently, there is a grand worldwide interest with the beneficial synergistic effects of natural supplements and their multiple ingredients as related to the single active fractions [15]. Semen freezing causes damage to spermatozoa leading to reduction in semen quality [16], but it is essential to conserve the supergenetic characters of our local breeds of buffalo. Semen freezing

is accompanied with cryodamage caused by overproduction of reactive oxygen species [17], so, the natural additive to the extender ameliorates the antioxidant effect and consequently improving the fertilizing potential of frozen spermatozoa [18] The findings of the existing study revealed that sperm motility was significantly kept high up to 11 days of chilling with the concentration 10 and 20% and also significantly kept high with the concentration MEEY 30 and 40% up to 10 days

if compared to the control. This indicates that it could be used in AI up to 11 days and 10 days of chilling. The use of MEEY had significantly improved post-thawing sperm motility% with the concentration 10 and 20% as compared to the control, while the other concentrations didn't result any effect. Additionally, MEEY had maintained alive sperm, intact spermatozoa membrane% and abnormal sperm % as good as the control with all the concentrations of MEE used in the current study. These results may be due to the strong antioxidant effect of *Moringa oleifera* extract at these concentrations through increasing the antioxidant action of glutathione, superoxide dismutase and catalase with reduced lipid peroxidation [19].

Then, it could be concluded that moringa as a natural additive at the concentration of 10 and 20% to semen extenders improved preservability in both chilled and frozen bull semen.

Ethical approval

The experimental design was approved and certified by the National Research Centre Medical Research Ethics Committee (Egypt) with an ethical certificate no. 17157.

Authorship

All the authors in a manuscript are responsible for the technical information communicated. All named authors have made an active contribution to the conception and design and/or analysis and interpretation of the data and/or the drafting of the paper and All have critically reviewed its content and have approved the final version submitted for publication.

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Conflict of interest

All authors claimed that there is not any conflict of interest concerning this manuscript.

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تقييم كفاءة حفظ السائل المنوي لطلانق الجاموس باستخدام مخفف التريس المحتوى على مستخلص المورينجا

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استهدفت الدراسة الحالية استكشاف تأثير مستخلص اوراق المورينجا اوليفيرا (*Moringaoleifera*) على الحيوانات المنوية للجاموس اثناء التبريد والتجميد. وقد تم تخفيف السائل المنوي المجمع للطلانق بمخفف التريس سترات الفركتورز المضاف اليه مستخلص المورينجا وتخصيصه فيسنة تركيزات هي صفر% (المجموعة الضابطة)، 10، 20، 30، 40، و 50% (حجم/حجم من مستخلص المورينجا : التريس سترات الفركتورز) ثم تم اضافة 20% من صفار البيض ومزج الخليط وتجميده عند -20°م لحين الاستخدام. وقد تم تبريد السائل المنوي المخفف عند 5°م وموازنته لمدة اربع ساعات. كما تم تحميل السائل المنوي في قصبية بوليفنيل 0.25 مللي. ثم تعريض القصبية لبخار النتروجين السائل لمدة عشر دقائق ثم غمرت في النتروجين السائل. وقد تم تقييم السائل المنوي المبرد والمجمد بعد اذابته من حيث الحركة ، الحيوية ، التشوهات ، صلابة الغشاء (%HOST) للحيوان المنوي. وباستخدام مخفف المورينجا ذات تركيزي 10 و 20% فقد تحسنت نسبة حركة الحيوانات المنوية. وعلى الجانب الاخر فقد حافظت جميع تركيزات مخفف المورينجا على حيوية الحيوان المنوي، وصلابة الغشاء وقللت من التشوهات مقارنة بالمجموعة الضابطة. ومن ثم ، فاننا نستخلص من هذه الدراسة ان استخدام مخفف مستخلص اوراق المورينجا بتركيزي 10 و 20% (وتحت ظروف الاستخلاص المتزامنة مع هذه الدراسة) كاضافة طبيعية الى مخفف السائل المنوي الاساسى قد ادى الى تحسين حفظ السائل المنوي لطلانق الجاموس المبرد والمجمد.