J. Animal and Poultry Prod., Mansoura Univ., Vol.10 (3): 67 - 71, 2019

Different Types and Levels of *Moringa oleifera* Leaf Extract as a Source of Antibiotics in Friesian Bull Semen Extender

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ABSTRACT

This study aimed to evaluate the effect of addition of aqueous or methanolic Moringa oleifera leaves extract (MOLE) at different levels (0, 100, 200 and 300 µg/ml) as antibiotic alternative to Friesian bull semen extender on sperm characteristics post-dilution, equilibration and thawed. The enzyme activity and antioxidant capacity of bull seminal plasma were determined in post-thawed semen. Five Friesian bulls (3-4 years old and 400-450 kg LBW) were used. Semen was collected from bulls by an artificial vagina twice a week for 5 weeks. After ejaculation semen was evaluated for mass motility and only the ejaculates of ≥70% percentage were pooled and diluted at 37^0C using citrate-egg yolk extender and divided into seven portions, control (E1), 100 (E2), 200 (E3), 300 (E4) µg aqueous MOLE /ml semen extender or 100 (E5), 200 (E6), 300 (E7) µg methanolic MOLE /ml semen extender. After semen dilution it was equilibrated at 5^0C for 4 h then it placed in liquid nitrogen at -196^0C. Frozen semen stored for two month was thawed at 37^0C for 30 sec. Semen samples were evaluated for characteristics including sperm motility, livability, abnormality, acrosome damage and hypo osmotic swelling test (sperm cells with curled tail) in diluted, equilibrated and thawed semen. Activity of AST, ALT, LDH, catalase and glutathione in post thawed samples seminal plasma were determined. The present results indicated that adding 300 µg of methanolic *Moringa oleifera leaves* extract to each 1 ml of citrate-egg yolk extender as an alternative to penicillin and streptomycin can improve quality of Friesian bull semen during different preservation stages.

Keywords: *bovine semen, Moringa oleifera, extraction, sperm function, enzyme activity.*

INTRODUCTION

Breeding program aims to maximize the number of offspring conceived with superior breeding bulls frozen semen using artificial insemination technique (Hallap *et al*., 2006). Artificial insemination (AI) is a modern way which used to increase the genetic improvement in cattle. In this technique, the superior genetic with high fertility bulls can breed numerous cows, thus it is essential tools for sustainable cattle production (Kastelic, 2013).

Freezing Friesian bull semen is one of the critical steps in AI (Allai *et al*., 2015) due to the oxidative damage of sperm cells by reactive oxygen species (ROS) during freezing process (Chatterjee and Gagnon, 2001) that may be decreased sperm motility and subsequently fertility (Bucak and Tekin 2007). Extender is a chemical medium used in semen dilution to allow more extensive use of individual semen sample to cover a large number of females. It supplies the spermatozoa with nutrients, provides buffers to against the changes in medium pH, control microbial contamination and preserve spermatozoa from cold shock or freezing storage temperature (Raheja *et al*., 2018). Semen extender had important role for animal breeders who used various types of semen dilution to extend and transport their semen to other breeders in another place around the world (Rehman *et al* 2013).

Spermatozoa are affected by bacteria both directly by it abiding to spermatozoa, decreasing their motility and producing acrosome damage, and indirectly by their residual toxins thus it is essential to added different antibiotic to semen extender to controlling bacterial activity (Andrabi, 2009; Mughal *et al*., 2017). *Moringa oleifera* (MO) is plant species belonged to *Moringaceae* (monogeneric) family that most widely cultivated in India, Bangladesh and Pakistan. Greeks, Romans, and Egyptians people are the first utilize of this plant parts due to its numerous potential uses. It acts as anti-bacterial, anti-inflammatory and anti-fungal (Sadek *et al*., 2013; Sokunbi *et al*., 2015).

The recent studies discovered the anti-bacterial benefits of MO leaves extracts (MOLE), which allow to using it in semen extender of cattle (Sokunbi *et al*., 2015; Okediran *et al*., 2017), buffaloes (El-Nagar *et al*., 2019) and rabbits (Ghodiah, 2016). Beside the anti-bacterial properties of MOLE, it is a good source of natural antioxidants because it contains two powerful antioxidant ascorbic acid and phenolic compounds (Huang *et al*., 2005). The extract of this plant leaves had effective scavenging of free radicals in-vitro (Oparinde and Atiba, 2014; Okediran *et al*., 2017).

More recent results of El-Nagar *et al*. (2019) indicated the successful usage of MOLE as a new promising extender or as an alternative to antibiotic in buffalo semen. Therefore, the current study aimed to evaluate the antibiotic potential of *Moringa oliefera* leaf as aqueous or methanolic extracts in replacing the conventional antibiotics in citrate egg yolk extender of Friesian bull semen.

MATERIALS AND METHODS

This study experimental work was carried out in cooperation between Animal Production Research Institute and Animal Production Department, Faculty of Agriculture, Tanta University.

Animal management:

Five healthy mature Friesian bulls aging 3-4 years and weighing 400-450 kg live body weight were used as semen donors. Bulls were in good health and clinically free from parasites. Animal were fed on daily ration according to NRC (1988) composed of 4 kg concentrate fed mixture (CFM), 3 kg clover hay and 4 kg rice straw, while fresh water was available at all day times over the collection period.

Semen collection:

Semen was collected before animal feeding using a France artificial vagina at 6-7 a.m. twice weekly for 5 weeks (50 ejaculates). The ejaculates were immediately taken in water bath at 37^0C to the laboratory for semen evaluation and freezing processes. Ejaculates with initial motility of ≥70% on each day of collection were pooled and divided into seven portions, then diluted in citrate egg yolk extender (CEY) with different levels of MOLE. The basal CEY extender was prepared with 5g sodium citrate ,58 mg glucose, 20 ml fresh egg yolk,7 ml glycerol, penicillin 1000

I.U. mL^{-1}, streptomycin 1mg mL^{-1} and completed with 73 ml distilled water according to Chaudhari and Mshelia (2002).

Preparation of *Moringa oliefera* leaves extracts:

Fresh MO leaves were harvested from Samanod city, Gharbiya government (located in the north part of Egypt, 30°N). Leaves of MO were dried for 48 hours in air under shade then in oven for 2 hours at 40°C, then grounded into a fine powder using electric blinder. The powder (20 g) was macerated in 20 ml ethanol (70%) or 20 ml distilled water for 48h and allowed to extract for 2 h using Soxhlte Device, then it lyophilized, weighted and preserved in freezer until usage (Ramluckan *et al*., 2014).

Experimental extenders:

Seven types of extenders were used in this study, including the basal control CEY supplemented with penicillin and streptomycin as conventional antibiotics (E1), 100 (E2), 200 (E3) or 300 (E4) µg of aqueous MOLE extract/ml extender or 100 (E5), 200 (E6) or 300 (E7) µg of methanolic MOLE extract/ml extender. Semen was extended at rate of 1:10 and the extended semen was kept in water both at 37°C. Semen characteristics were evaluated in post-diluted, post-equilibrated (at 4-5°C for 4 h) and post-thawed semen, while enzyme activities were determined only in post-thawed semen seminal plasma.

Evaluation of sperm characteristics:

Semen in each treatment was evaluated after dilution, equilibration and thawing (n=6) for the percentage of progressive motility (Amann and Hammerstedt, 1980), livability (Hackett and Macpherson, 1965), abnormality (Blom, 1983) and acrosome status (Yanagimachi, 1982).

Hypo-osmotic swelling test was done at osmolarity level of 50 mOsm/l for 30 min to access the percentage of sperm with curled tail (El-Sherbieny, 2004). Frozen semen for at least one month was thawed at a rate of 37°C for 30 s.

Enzyme activity:

Seminal plasma of post-thawed semen was isolated by semen centrifugation at 4000 rpm for 15 min, then stored at -20°C until analysis. Activity of amino-transaminases (AST and ALT), and lactic dehydrogenase (LDH) was determined in seminal plasma by spectrophotometer (JENWAY-6405UV/Vis) using commercial kits (Salucea Netherlands) according to Young (1990). Antioxidant enzyme activity of catalase (Cohen *et al*., 1970) and Glutathione peroxidase (Bell *et al*., 1986) was also determined.

Statistical analysis:

The obtained data were statistically analyzed using compare means using one-way ANOVA design of SPSS program (2013) version 20. Duncan multiple range test was used to test the differences between experimental treatment means (Duncan, 1955) at $P<0.05$. The percentage values of sperm characteristics 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

RESULTS

Sperm characteristics:

In diluted semen:

All sperm characteristics (Table 1) in post-diluted semen were significantly ($P<0.05$) affected by extender type. The present results showed that adding aqueous MOLE only at a level of 300 µg/ml CEY extender (E4) or all levels of methanolic MOLE (E5-E7) significantly ($P<0.05$) improved all sperm characteristics in diluted semen as compared to control extender free from MOLE (E1) in terms of increasing percentages of sperm motility and livability, higher response of sperm cells to HOS-t (curled tail%) and decreasing percentages of abnormality and acrosomal damage of spermatozoa. In addition, increasing level of methanolic MOLE at a level of 300 µg/ml showed additionally significant ($P<0.05$) improvement in all sperm characteristics in diluted semen as compared to other levels of methanolic MOLE (E5 and E6) and even from aqueous MOLE at a level of 300 µg/ml (E4). It is of interest to note that aqueous MOLE at levels of 100 and 200 µg/ml maintained all sperm characteristics in E2 and E3 as in E1 with conventional antibiotics.

These results can indicated that MOLE supplementation in Friesian bull semen extender has beneficial effect on sperm function after semen dilution, being good for aqueous MOLE at level of 300 µg/ml, while the best results were obtained from supplementation of methanolic MOLE at a level of 300 µg/ml CEY extender.

Table 1. Effect of extender with different types and levels of MOLE on sperm characteristics in post-diluted Friesian bull semen.

Item	Sperm characteristics (%)				
	Motile	Live	Abnormal	Acrosome damage	Curled tail
CEY + antibiotics (E1)	67.50±1.34^{d}	70.50±1.34^{e}	25.40±0.83^{a}	26.40±0.69^{a}	60.60±1.42^{c}
CEY+100 µg aMOLE (E2)	69.00±1.45^{cd}	73.00±1.45^{de}	23.10±0.75^{b}	26.60±0.54^{a}	61.90±1.26^{c}
CEY+200 µg aMOLE (E3)	69.00±1.00^{cd}	74.00±1.00^{cde}	23.00±0.65^{b}	25.80±0.49^{a}	61.90±0.95^{c}
CEY+300 µg aMOLE (E4)	71.50±1.67^{bc}	76.50±1.67^{cd}	17.80±0.81^{c}	22.60±0.45^{b}	69.00±1.58^{b}
CEY+100 µg mMOLE (E5)	75.00±1.05^{b}	81.00±1.05^{b}	17.70±0.62^{c}	22.00±0.47^{b}	68.80±0.90^{b}
CEY+200 µg mMOLE (E6)	73.00±1.11^{b}	77.00±1.11^{c}	19.30±0.58^{c}	19.40±0.58^{c}	65.80±1.38^{b}
CEY+300 µg mMOLE (E7)	78.50±1.07^{a}	85.50±1.07^{a}	14.30±0.65^{d}	17.40±0.45^{d}	72.70±1.40^{a}

a, b…….e: Means in the same column with different superscripts differ significantly at P<0.05.
aMOLE: Aqueous extract. mMOLE: Methanolic extract.

In post-equilibrated semen:

All sperm characteristics (Table 2) in post-equilibrated semen were significantly ($P<0.05$) affected by extender type. It is worthy noting that all sperm characteristics in equilibrated semen showed similar trend to those observed in post-diluted semen reflecting maintaining all sperm characteristics in semen diluted with CEY extender supplemented with 100 or 200 µg/ml and benefits of all levels of methanolic MOLE on sperm function after cooling at 4-5^{o}C for 4 hours as equilibration period, being the best from supplementation of methanolic MOLE at a level of 300 µg/ml CEY extender.

Up to these stages of semen processing for cryopreservation, both types and levels of MOLE showed acceptable results on all sperm characteristics, indicating the successful usage of aqueous or methanoic MOLE as an alternative to conventional antibiotics in CEY extender for semen preservation in refrigerator at cool temperature for at least 4 hours.

Table 2. Effect of extender with different types and levels of MOLE on sperm characteristics in post-equilibrated Friesian bull semen.

Item	Sperm characteristics (%)				
	Motile	Live	Abnormal	Acrosome damage	Curled tail
CEY + antibiotics (E1)	62.50±1.34^{c}	65.50±1.34^{d}	29.00±0.61^{ab}	30.80±0.83^{a}	55.50±1.35^{c}
CEY+100 µg aMOLE (E2)	63.50±1.07^{c}	67.50±1.07^{cd}	27.70±0.98^{b}	29.00±0.45^{b}	56.60±1.33^{c}
CEY+200 µg aMOLE (E3)	65.50±1.17^{c}	70.50±1.17^{c}	29.70±0.47^{a}	28.40±0.58^{b}	58.50±1.02^{c}
CEY+300 µg aMOLE (E4)	69.00±1.25^{b}	74.00±1.25^{b}	22.80±0.88^{d}	24.40±0.44^{c}	62.90±1.45^{b}
CEY+100 µg mMOLE (E5)	70.50±1.17^{b}	74.50±1.17^{b}	24.70±0.47^{c}	22.80±0.49^{d}	64.50±1.02^{b}
CEY+200 µg mMOLE (E6)	70.50±0.90^{b}	76.50±0.90^{b}	20.40±0.50^{e}	21.00±0.33^{e}	63.80±0.84^{b}
CEY+300 µg mMOLE (E7)	75.00±1.05^{a}	80.00±1.05^{a}	15.70±0.56^{f}	19.00±0.47^{f}	69.00±0.95^{a}

a, b……f: Means in the same column with different superscripts differ significantly at P<0.05.
aMOLE: Aqueous extract. mMOLE: Methanolic extract.

In post-thawed semen:

All sperm characteristics (Table 3) in post-thawed semen were significantly (P<0.05) affected by extender type.

The obtained results cleared that adding aqueous MOLE only at a level of 300 µg/ml CEY extender (E4) or all levels of methanolic MOLE (E5-E7) significantly (P<0.05) increased percentages of motility, livability and response to HOS-t of spermatozoa, while decreased sperm abnormality in thawed semen as compared to control extender free from MOLE (E1). However, all types and levels of MOLE had pronounced and significant (P<0.05) effect on decreasing acrosomal damage percentage as compared to control, being significantly the lowest for semen extended with CEY extender supplemented with methanolic MOLE at a level of 300 µg/ml.

These results indicated potential effect of supplementation CEY extender with aqueous or methanolic MOLE, as alternatives to natural antibiotics, on cryopreservation of Friesian bull semen, in particular with methanolic MOLE at a level of 300 µg/ml CEY extender.

Table 3. Effect of extender with different types and levels of MOLE on sperm characteristics in post-thawed Friesian bull semen.

Item	Sperm characteristics (%)				
	Motile	Live	Abnormal	Acrosome damage	Curled tail
CEY+antibiotics (E1)	42.50±1.71^{d}	46.50±1.71^{c}	42.80±1.66^{a}	38.40±1.78^{a}	35.50±1.81^{c}
CEY+100 µg aMOLE (E2)	44.50±1.38^{d}	49.50±1.38^{c}	41.40±1.42^{a}	35.00±0.67^{b}	37.40±1.42^{c}
CEY+200 µg aMOLE (E3)	46.00±1.25^{cd}	50.00±1.25^{c}	40.70±1.58^{a}	32.80±0.98^{bc}	39.10±1.43^{c}
CEY+300 µg aMOLE (E4)	50.00±1.49^{bc}	56.00±1.49^{b}	35.50±1.39^{b}	30.20±0.55^{cd}	44.00±1.42^{b}
CEY+100 µg mMOLE (E5)	51.50±1.30^{b}	56.50±1.30^{b}	35.40±1.75^{b}	29.60±0.67^{d}	45.40±1.36^{b}
CEY+200 µg mMOLE (E6)	54.00±1.63^{b}	58.00±1.63^{b}	27.00±1.05^{c}	27.50±0.52^{d}	46.80±1.79^{b}
CEY+300 µg mMOLE (E7)	59.00±1.94^{a}	66.00±1.94^{a}	23.00±1.11^{c}	24.40±1.03^{e}	53.10±2.18^{a}

a, b……e: Means in the same column with different superscripts differ significantly at P<0.05.
aMOLE: Aqueous extract. mMOLE: Methanolic extract.

Enzyme activity in seminal plasma of post-thawed semen:

Data in Table (4) showed that the extender type affected significantly (P<0.05) on the activity of metabolic enzymes (AST, ALT and LDH) and concentration of antioxidant enzymes (catalase and glutathione).

Table 4. Enzyme activity and antioxidant capacity in seminal plasma of post-thawed Friesian bull as affected by type and level of MOLE in CEY extender.

Item	Enzyme activity				
	AST (IU/L)	ALT (IU/L)	LDH (IU/L)	Catalase (mg/dl)	Glutathione (mg/dl)
CEY+antibiotics (E1)	75.20±1.11^{a}	50.20±1.39^{a}	364.80±2.37^{a}	4.14±0.48^{b}	8.91±0.62^{d}
CEY+100 µg aMOLE (E2)	70.40±2.34^{ab}	49.80±1.24^{a}	358.80±1.93^{ab}	4.33±0.30^{ab}	9.33±0.46^{cd}
CEY+200 µg aMOLE (E3)	70.60±1.81^{ab}	50.60±1.03^{a}	356.40±1.21^{b}	4.50±0.37^{ab}	9.55±0.78^{cd}
CEY+300 µg aMOLE (E4)	63.40±1.57^{c}	44.80±1.24^{bc}	347.80±2.13^{c}	4.90±0.67^{ab}	10.29±1.16^{cd}
CEY+100 µg mMOLE (E5)	67.00±1.58^{bc}	45.40±1.12^{bc}	343.40±2.42^{c}	5.13±0.53^{ab}	11.40±0.56^{bc}
CEY+200 µg mMOLE (E6)	64.00±2.65^{c}	46.80±1.69^{b}	338.40±2.62^{d}	5.59±0.49^{ab}	13.23±0.63^{ab}
CEY+300 µg mMOLE (E7)	57.00±2.37^{d}	41.80±1.43^{c}	328.00±2.30^{e}	6.04±0.73^{a}	13.88±0.75^{a}

a, b……e: Means in the same column with different superscripts differ significantly at P<0.05.
aMOLE: Aqueous extract. mMOLE: Methanolic extract.

Activity of AST, ALT and LDH significantly (P<0.05) reduced in seminal plasma of semen extended with MOLE as aqueous at a level of 300 µg/ml or all levels of methanolic extract type, being the lowest in seminal plasma of semen extended with the highest level of methanolic MOLE (300 µg/ml). However, concentration of catalase significantly (P<0.05) increased with methanolic MOLE (300 µg/ml), while glutathione concentration significantly (P<0.05) increased with all levels of methanolic MOLE only.

Generally, supplementation of CEY extender with methanolic MOLE at a level of 300 µg/ml showed significantly the best results concerning marked reduction in AST, ALT and LDH activity, and increasing antioxidant capacity in seminal plasma of Friesian bull semen (Table 4)

DISCUSSION

Quality and composition of the semen extender are fundamental for successful AI. Semen with standards of motility and morphology, and the minimum bacterial contamination is qualified as good. In bacterial contaminated semen used in AI, sperm viability decreases, the risk of pathologies in the female genital tract increases, and subsequently fertility of inseminated animals is impaired (Salamon and Maxwell, 2000). There are many efforts in order to substitute several sources of antibiotics such as penicillin with streptomycin in egg yolk-based extenders by other antibiotics (Azawi and Ismaeel, 2012). Recently, there are many natural antibacterial compounds such as MOLE able to minimizing the bacterial contamination of semen extenders used for semen cryopreservation in buffaloes (El-Nagar *et al*, 2019). The present results aimed to evaluate the potential effect of MOLE as aqueous or methanolic extracts as a natural anti-bacterial and anti-oxidant in CEY extender of cryopreserved Friesian bull semen.

Controlling the microbial contamination and preservation of spermatozoa from cold shock or freezing temperature is essential for obtaining best results following AI (Raheja *et al*., 2018). Also, De Jarnette *et al*. (2004) confirmed that the antibiotics addition to semen extender is one of the major advances to improve fertility with using artificial insemination technique. According to the obtained results in our study, using MOLE as aqueous extract at levels of 100 or 200 µg/ml maintained all sperm characteristics in semen post dilution, equilibration and thawing, as well as activity of AST, ALT and LDH, and concentration of catalase and glutathione in seminal plasma of post-thawed semen, reflecting similar effect of using conventional antibiotics and aqueous MOLE at levels of 100 or 200 µg/ml, beside the wide variation in price of each supplement. It is of interest to note that, the increase in percentage of normal morphological sperm is an indicator for reduced the activity of microorganisms in semen (Sokunbi *et al*., 2015).

However, aqueous MOL at 300 µg/ml and all levels of methanolic MOLE exhibited significantly additional improvement in all above traits as compared to conventional antibiotics in CEY extender. These results may be attributed to the method of MOL extraction, whereas methanolic extraction may result in release of additional beneficial compounds as compared to aqueous extraction. Extracts of MOL may prevent the impaired effects of bacteria in semen extender. Bacteria abiding to sperm cells decrease motility and damage acrosome, beside their residual toxins on spermatozoa (Andrabi, 2009; Mughal *et al*., 2017). It is of interest to note that ethanolic MOLE describable high activity than the aqueous one compared with different industrial antibiotics like ciprofloxacin, chloramphenicol and cotrimoxazole (Doughari *et al*., 2007). In accordance with the present results, El-Nagar (2017) use aqueous MOLE as extender in dilution and freezing Friesian bull semen, indicating significant effect for MOLE as a source of antibiotics and antioxidants. Also, Dowidar *et al*. (2018) indicated that all sperm characteristics were improved by using ethanolic MOLE as a semen extender or as a replacement of antibiotic for buffalo semen extension.

These findings may indicate the anti-microbial effect of aqueous or methanolic MOLE depending on the level of supplementation and extract type. In this respect, Peixoto *et al*. (2011) indicated that aqueous MOLE contain components with antibacterial activity able to inhibiting the gram-positive and negative bacteria growth. Also, Sofidiya *et al*. (2006) and Ogbunugafor *et al*. (2011) mentioned that the medicinal effects of MOLE were attributed to their antioxidants compounds suppressing ROS formation and scavenging the free radicals producing by lipid peroxidation. Also, Luqman *et al*. (2012) indicated significant effect for MOLE on activation of antioxidant enzymes and inhibiting the oxidases.

CONCLUSION

The present results indicated that adding 300 µg of methanolic *Moringa oleifera leaves* extract to each 1 ml of citrate-egg yolk extender as an alternative to penicillin and streptomycin can improve quality of Friesian bull semen during different preservation stages.

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أنواع ومستويات مختلفة من مستخلص أوراق المورنجا كمصدر للمضادات الحيوية في مخفف السائل المنوي لطلائق الفريزيان

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إستهدفت هذة الدراسة تقييم تأثير إضافة المستخلص المائي أو الميثانول لأوراق المورنجا بمستويات مختلفة (صفر , 100 , 200 , 300 ميكروجرام/مل مخفف) كبديل للمضادات الحيوية في مخفف السائل المنوي لطلائق الفريزيان على خصائص الحيوانات المنوية بعد التخفيف , الموازنة والإسالة. تم تقدير النشاط الانزيمي وقدرة مضادات الأكسدة لبلازما السائل المنوي بعد الاسالة. تم إستخدام خمس طلائق فريزيان عمر 3-4 سنوات ووزن حي 400-450 كجم وتم تجميع السائل المنوي منها مرتين أسبوعيا بإستخدام مهبل صناعي لمدة خمس أسابيع. تم تقييم السائل المنوي بعد القذفللحركة التقدمية وتم خلط القذفات ذات الحركة الجماعية أكبر من أو يساوي 70% وتم تخفيفها على درجة حرارة 37°م باستخدام مخفف السترات – صفار البيض وقسمت إلى 7 معاملات كنترول (م1) , 100 (م2) , 200 (م3) , 300 (م4) ميكروجرام مستخلص مائي لأوراق المورنجا لكل مل مخفف أو 100 (م5) , 200 (م6) , 300 (م7) ميكروجرام مستخلص الميثانول لأوراق المورنجا. بعد التخفيف تم عمل فترة موازنة على درجة حرارة 5°م لمدة أربع ساعات ثم تم وضعها في النيتروجين السائل على درجة حرارة -196°م. تم إسالة السائل المنوي المجمد على درجة حرارة 37°م لمدة 30 ثانية وذلك بعد التخزين لمدة شهرين. تم تقييم السائل المنوي لصفات حركة الحيوانات المنوية والحياتية والشواذ وذات الأكروسوم الغير سليم والملتوية الذيل للسائل المنوي المخفف وبعد الموازنة وبعد الاسالة. تم تقدير النشاط الانزيمي لكل من AST , ALT , LDH , Catalase و Glutathione في عينات السائل المنوي المسالة. أظهرت النتائج أن إضافة 300 ميكروجرام مستخلص الميثانول لأوراق المورنجا لكل مل مخفف سترات – صفار البيض كبديل للبنسلين والاستربتومايسين يمكن أن يحسن جودة السائل المنوي لطلائق الفريزيان خلال فترات الحفظ المختلفة.