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Freezing and Fertilizing Abilities of Summer Semen of Egyptian Buffalo *(Bubalus bubalis)* Bulls Using Moring Extract as Antibiotic or as a New Promising Extender

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ABSTRACT

Semen collection is the business for various artificial insemination organizations, hence the management of entire process of collection is quite critical. Therefore, the trend now is to design procedures or refine methodologies so as to maximize the production of good quality semen without discarding too many poor quality ejaculates. The present work aimed to study the effect of seasons (winter vs. summer) on buffalo semen characteristics, and evaluating the freezing ability and fertilizing ability of buffalo semen collected in summer and extended with ethanolic *Moringa oleifera* leaf extract (eMOLE) natural antibiotics and antioxidant in extender. Semen was collected twice weekly by artificial vagina from four healthy matured buffalo bulls (400-450 kg body weight 4-5 years old) during winter and summer seasons for 10 weeks in winter (Dec-February) and in summer (July-August). Ejaculates were evaluated for some semen characteristics during both seasons (1st experiment). In the 2nd experiment, only ejaculates of ≥70% as initial sperm motility were extended by citrate egg yolk (CEY), eMOLE as anti-biotics in CEY (CEYm), eMOLE and cryopreserved in liquid nitrogen (-196°C). Post-thawed semen was evaluated physically and chemically, and pregnancy rate as done as a fertility study. Results show that ejaculate volume, and percentages of live, acrosome integrity, and membrane integrity, increased ($P<0.05$) in summer compared with winter. However, the effect of season on semen density, and motility and abnormal sperm percentages was not significant. Extension of semen by eMOLE resulted improving ($P<0.05$) percentage of sperm motility, livability, abnormality and intact acrosome in post-thawed semen. Also, enzyme activities (AST, ALT and LDH) reduced to the minimal levels in seminal plasma of post-thawed semen with eMOLE. Replacing antibiotics in CEY extender by eMOLE (4 ml/100 ml) had no significant effect on all sperm characteristics and enzyme activity. Semen extended with eMOLE showed significantly ($P<0.05$) higher fertility rate (83.3%) than that extended with CEY or CEYm (66.6%, $P<0.05$). In conclusion, buffalo semen collection during summer months (July-August) in Egypt can be used for insemination in association with better quality than that of winter semen. From the economical point of view, eMOLE is considered as a new promising extender and as an alternative for anti-biotic and efficient antioxidant in extenders of buffalo semen collected and cryopreserved during summer.

Keywords: *Buffalo semen, season, Moringa, extender, anti-biotics, antioxidant, cryopreservation.*

INTRODUCTION

Buffalo is known as "Black Gold" due to its contribution to economy, as a source for milk, meat, hide and bone all over the world. There are many considerations for the importance of buffalo and realizing the need of research for its improvement (Michelizzi *et al*., 2010).

Conception rate in buffaloes under artificial insemination (AI) system was reported to 41.4 – 60.8% (Sosa *et al*., 2003), which may be due to season of the year (Bhavsar *et al*., 1989), type of used extender (Sosa *et al*., 2003) and low percentage of viable spermatozoa after freezing (Barkawi *et al*., 2006). Conflicted results were reported on the effect of season of the year on semen quality of buffalo bulls (Hussain, *et al* 2002; Barkawi *et al*., 2006).

Improvement of buffalo semen cryopreservation requires a better understanding the properties of the currently used extenders. The first aim of sperm freezing protocols is to prevent lethal ice crystal formation and to reduce membrane damage during and after cryopreservation. Freezing of semen was reported to have an adverse effect on the intactness of plasma membrane and acrosome of spermatozoa, leading to poor fertility of the cryopreserved spermatozoa (Nagy *et al*., 2004; Barkawi *et al*., 2006). Using different types of antibiotics in semen diluent is important to control the harmful effect of bacteria to spermatozoa for maximized the AI results (Akhter *et al*., 2007; 2008; Mughal *et al*., 2017).

There are global problems of multiple antibiotics resistance (Albuquerque *et al.,* 2007). Antimicrobial resistance among enteric pathogens is becoming a matter of serious concern (Mahmood *et al.,* 2010) and poses a great threat to global human health. As a result, it has become imperative to discover and invent new types of semen diluents based on natural extracts according to guidelines of CSS (2011) as recently reported by Dowidar *et al*. (2018). The composition of the extender in which the semen is diluted before freezing is one of the main factors that influence the success of cryopreservation (De Leeuw *et al*., 1993; Dhami *et al*., 1994; Woelders *et al*., 1997). High content of polyunsaturated fatty acids in the plasma membrane and a low level of antioxidant in the sperm cytoplasm make them susceptible to oxidative stress during preservation (Aitken *et al*., 1998; Chatterjee and Gagnon, 2001). A balanced generation of reactive oxygen species (ROS) and antioxidant enzymes is associated with normal physiological functions. An unbalanced, excessive production of ROS and decreased level of antioxidant enzymes caused decreased sperm motility and viability and increased sperm defects (Aitken and Baker, 2004; Sikka, 2004).

Moringa Oleifera (MO), family *Moringaceae*, is native to Arabia, Africa, India, Southeast Asia, South America and the Pacific and Caribbean Islands (Iqbal and Bhanger, 2006). Leaves of MO has many different chemical components, including crude fiber, reducing sugars, resins, alkaloids, flavnoids, organic acids, sterols, tannins, saponins, and proteins. Moringa has been found to be a good source of polyphenols and antioxidants (Mishra *et al.,* 2011). MO oil and its micronutrients contain anti-tumor, anti-oxidant, anti-epileptic, anti-diuretic, anti-inflammatory, hepato-protective and anti-diabetic properties (Hsu *et al*., 2006; Sreelatha and Padma, 2010). Also, MO contains compounds help in protection against oxidative changes such as fundamental antioxidants and phenols (Siddhuraju and Becker, 2003). The anti-oxidative properties of MO and its ability to increase the anti-oxidant capability were reported by Saalu *et al*. (2011).

These phenolic compounds in MO are act as free radical terminators and play important role in impedance of oxidative lipid degradation (Pourmorad *et al*., 2006; Lukacinova *et al*., 2008; Pakade *et al*., 2013).

Recently, MO was used by several investigators in extenders of bovine (Sokunbi *et al*., 2015), sheep (El-Harairy *et al*., 2016) and rabbit (Ghodaia, 2016) as an antioxidant or as alternative to antibiotics. There is paucity of information on the assessment of the antibacterial property of eMOLE in buffalo bull semen extender (Sokunbi *et al*., 2015). The current work was conducted to 1) evaluate the season effect (winter vs. summer) on buffalo semen characteristics, and 2) evaluating the freezing and fertilizing abilities of post-thawed buffalo semen collected in summer season and extended with ethanolic MO leaf extract (eMOLE) as a new extender (eMOLE) or as alternative for anti-biotics in citrate extender of cryopreserved buffalo semen.

MATERIALS AND METHODS

The present study was conducted in the Department of Biotechnology, Faculty of Agriculture, Al-Azhar University. The experimental work was carried out at Animal Production Research Station, El-Gemmezah, Gharbiya Governorate, belonging to Animal Production Research Institute (APRI), Egypt, during December 2015 - December, 2016.

Climatic condition:

During winter (December-February) and summer (June-August) season, degrees of ambient temperatures (AT, °C) and percentages of relative humidity (RH, %) were obtained from meteorological data of El-Gemmezah region, while values of temperature-humidity index (THI) was calculated according to LPHSI (1990). These data are illustrated in Table 1. The obtained values of THI indicated absence of heat stress in winter (THI <72) and severe heat stress (THI 74 to <78) in summer.

Table 1. Means of maximum ambient temperatures (AT, °C), relative humidity (%) and calculated temperature-humidity index (THI) during winter and summer months.

Season	AT (°C)	RH (%)	THI	Photoperiod (h)
Winter (Dec.-Feb.)	21.66±0.27	58.79±1.18	58.02	12.84
Summer (June-Aug.)	31.46±0.46	58.54±0.95	78.80	14.12

Animals:

Four healthy matured buffalo bulls (400-450 kg body weight 4-5 years old) were used as semen donors in two experiments. All bulls were typically normal and clinically in healthy condition. Bulls were kept in semi-open yard with 75% shed all the day. Bulls were trained to serve the artificial vagina for semen collection two months before the start of each experiment.

Feeding and management:

During semen collection period, each bull was daily fed on 6 kg concentrate feed mixture (CFM), 4 kg rice straw and 15 kg Egyptian clover (*Trifolium alexandrinum*) during winter or 16 kg Darawa (green maize) during summer. The CFM consisted of 25% undecorticated cotton seed cake, 15% rice polish, 20% wheat bran, 35% yellow maize, 3% calcium carbonate, 1% mineral mixture and 1% sodium chloride, based on the APRI requirements for adult buffalo bulls. Feeds were individually offered at 8 a.m. and 3 p.m. Drinking water and blocks of minerals were found at all times.

Collection of semen:

Semen ejaculates were collected for 10 weeks during winter (Dec.-Feb.) and summer (Jul.-Aug.) for the 1st experiment, and during summer (July-August) for the 2nd experiment. Semen was collected at 7-8: a.m. from four buffalo bulls, using a sterile artificial vagina. Semen ejaculates were collected from each bull twice a week. The collected ejaculates were transferred immediately to the laboratory in water bath (37°C) for respective evaluation.

First experiment:

This experiment was carried out to compare the physical semen characteristics of buffaloes in winter and summer seasons. Each ejaculate was evaluated for the following variables: ejaculate volume, semen density, and percentages of progressive motility, livability, abnormality, and acrosome and membrane integrities of spermatozoa.

Second experiment:

After collection of semen, only ejaculates with mass motility more than 70% were pooled and extended with citrate egg-yolk (CEY) extender (T1, control), CEY with ethanolic *Moringa oleifera* leaves extract (eMOLE) as antibiotics (T2) and eMOLE as an extender without antibiotics (T3).

The eMOLE was prepared by Egyp. Sci. Soci. Moringa, National Research Center, (Ugwu Okechukwu *et al*., 2013). Each type of extender (Table 2) was warmed in water bath at 37°C to extention of semen at a rate of 1:20.

Table 2. Composition of different extender types used in the 2nd experiment.

Extender Type*	Fructose (g)	Sodium citrate (g)	Streptomycin sulphate (mg)	Penciline (IU)	eMOLE (ml)
T1 (CEY)	0.5	2.9	100.00	100.000	-
T2 (CEYm)	0.5	2.9	-	-	4
T3 (eMOLE)	0.7	-	-	-	14

*** Components of extender were dissolved in distilled water up to 100 ml, and then egg yolk (16 ml) and glycerol (8 ml) were added to a mixture of 76 ml.**

After dilution, semen was gently mixed with each extender, warmed (37°C) in a water bath, stored at 5°C for 4 hours as a an equilibration period. Then, semen was packaged in 0.25 ml French straws and frozen in liquid nitrogen container (-196°C) for one month. Thawing rate of the semen was 37°C for 30 seconds before evaluation.

Semen evaluation:

Semen ejaculate volume was recorded, then semen density as visual appearance of each ejaculate was scored at a scale from 0 to 3 after the method of Zemjanis (1970), while percentages of progressive sperm motility (Ewuola and Egbunike, 2010), live sperm (Campell *et al*., 1956), abnormal perm (Ewuola and Egbunike, 2010), acrosome integrity and membrane integrity were determined in fresh semen (experiment 1). However, only percentages of progressive sperm motility, live sperm, abnormal sperm acrosome integrity (Jankovicova *et al*., 2006) and membrane integrity were determined in diluted, equilibrated and thawed semen (experiment 2).

Plasma membrane integrity of spermatozoa was assessed using Hypo-Osmotic Swelling test (HOS-t) as described by Khan and Ijaz (2008), in term of curled tail percentage recorded at osmolarity level of 190 mOsmol/l for 45 min.

Enzyme activity in seminal plasma:

In seminal plasma of post-thawed semen, activity of AST, ALT and LDH was determined by commercial kits (Salucea Netherlands) and spectrophotometer (JENWAY-6405 UV/Vis) according to Young (1990).

Pregnancy rate

Total of 36 cyclic buffalo cows taken from Al-Gemmizah Research Station herd were were synchronized to estrus by i.m. single injection with 3 ml Estrumate (PGF2α-Essex Animal Helth Friesoythe, Germany) per cow and divided into three grops. All animals exhibited estrus activity within 48-72 h, then animals in heat were artificially insemination two times at 12 h-interval.

Buffalo cows artificially inseminated with semen thawed at 37°C for 30 seconds according to Salisbury *et al*. (1978). Pregnancy was diagnosed by rectal palpation on day 50 post-insemination to determined pregnancy rate.

Statistical analysis:

The obtained data for both 1st and 2nd experiment were statistically analyzed using the procedures of SPSS (2013) to study the effect of season (one way analysis) in the first experiment as well as the effect of extender type. Only for the 2nd experiment, Duncan Multiple Range Test (Dancan, 1955) was used to identify the significant differences among means. Chi-square test was used for data of pregnancy rates.

RESULTS AND DISCUSSION

Experiment 1:

Semen quality as affected by season:

Season had significant effect on ejaculate volume ($P<0.05$), and percentage of live sperm, acrosome integrity and membrane integrity ($P<0.001$), being better in summer than in winter season. However, semen density and percentages of progressive motility and motility of spermatozoa tended to be insignificantly ($P\leq0.05$) better in summer than I winter (Table 3).

Table 3. Physical semen characteristics of buffalo bulls during winter and summer.

Characteristics	Winter	Summer	P-value
Ejaculation volume (ml)	3.67±0.62	4.20±0.68	0.016*
Semen density (score: 0-3)	2.43±0.35	2.50±0.40	0.616
Progressive motility (%)	70.34±4.60	72.34±4.41	0.186
Live sperm (%)	69.84±3.02	83.21±2.36	0.000***
Sperm abnormality (%)	15.56±2.33	13.25±6.04	0.172
Acrosome integrity (%)	15.06±2.13	12.31±1.24	0.000***
Membrane integrity (%)	67.28±3.70	90.62±2.53	0.000***

*** Significant at P<0.05. *** Significant at P<0.001.**

The present values of all semen characteristics of Egyptian buffalo bulls during summer and winter season are within normal ranges of ejaculate volume (Gokhale *et al*., 2003; Nandre, 2007), semen density score (Osman, 1996; Shelke and Dhami, 2001), progressive sperm motility percentage (Bhatt *et al*., 2004; Alvai-Shoushtari *et al*., 2009), live sperm percentage (Nandre, 2007; Alvai-Shoushtari *et al*., 2009), sperm abnormality percentage (Bhavsar, 1987; Dhami *et al*., 1998; Singh *et al*., 2014), acrosome integrity percentage (Mandal *et al*., 2000), and sperm cell membrane integrity (Barkawi *et al*., 2006; El-Sheshtawy *et al*., 2008).

There are several factors affecting sperm characteristic such as, animal breed, individuals, and method and frequency of semen collection (Tomar and Singh, 1996).

In accordance with concerning results, several authors indicated higher ejaculate volume of Murrah buffalo bull in summer than in winter (Mandal *et al*., 2000; 2003), while an opposite trend was observed in Egyptian buffaloes (Osman, 1988; Barkawi *et al*., 2006) and Indian buffalo bulls (Sidhu and Gill, 1994). It is well known that increasing ejaculate volume in summer may reflect higher functional status of the accessory sex gland and higher testosterone concentration, since major part of semen contributed by different accessory glands secretion (Abdel-Khalek *et al*., 2001). It is of interest to observe that increasing semen volume in summer was associated with improving most sperm characteristics. In this context, Laing *et al*. (1988) stated that ejaculate volume is more indicative for fertility and bulls of high fertility produced greater semen volume.

The observed similarity in semen density may indicate similarity also in sperm cell concentration in both seasons. In Egyptian buffaloes, Osman (1988; 1996) reported no seasonal effect was found on density of semen, while Barkawi *et al*. (2006) revealed that semen density was significantly higher (2.3) in the winter than in summer (2.2).

In comparable with the present results, several authors reported higher percentage of sperm motility in summer than in winter season in Egyptian (El-Azab, 1980) and Indian (Sidhu and Gill, 1994; Mandal *et al*., 2000) buffalo bulls. Contrary, Osman (1988; 1996) indicated lower percentage of individual motility (65.7%) in summer than in winter season (69.9%).

The observed increase of live sperm percentage in summer than in winter was reported in buffaloes by Dixit *et al*. (1985), Agrawal *et al*. (1991) and Mandal *et al*. (2000), who found that live sperm percentage ranged 88.3-93.0% in summer versus 76.1-88.5% in winter, while an adverse situation was reported by Mohamed (1981) and Osman (1988; 1996) in Egyptian buffalo.

The present insignificant effect of season on sperm abnormality agreed with that reported by Mohamed (1981) and Osman (1988) on Egyptian bulls, and by Huuer *et al*. (1987) on buffalo bulls, who found higher percentage of sperm abnormalities in winter (25.2%) than in summer (14.8%). Sperm morphology provides information for the efficiency of spermatogenesis (Waberski *et al*., 1994). Also, Tsakmakidis *et al*., (2010) stated that boar fertility after artificial insemination with freshly diluted semen can be predicted based upon the evaluation of sperm morphology and chromatin integrity.Accordingly, the present trend of sperm abnormality percentage may indicate slight effect of season on spermatogenesis in buffalo bulls in Egypt.

Increasing the percentage of acrosome integrity of Egyptian buffalo spermatozoa in summer was reported in Murrah buffalo bulls (Mandal *et al*., 2000), bovine bulls (Barth and Oka, 1989; Thundathil *et al*., 2000).

The integrity of plasma membrane of sperm cells is a better predictor of the fertilizing capacity of spermatozoa (Vazquez *et al*. 1997; Correa and Zavos, 1995). Plasma membrane integrity is of utmost relevance of fertilizing ability (Azam *et al*., 1998).The plasma membrane functional status is of particular importance for sperm viability and maintenance of fertilizing capability (Clulow *et al*., 2008). The effect of season on plasma membrane integrity may be due to the change in total protein percentage in plasma membrane (Manjunth and Therien, 2002). Also, breed of the

animal, method used for evaluation, variation in the tonicity and pH of the stain (Campbell *et al*., 1956) could be responsible for such discrepancy.

In general, the significant effect of season on livability and integrity of acrosome and plasma membrane, being better in summer than in winter may reflect higher functional status of the accessory sex gland, higher testosterone level and spermatogenesis in summer (hot climate) than in winter (cold climate). In mammals spermatogenesis is totally dependent upon testosterone. It is produced by the Leydig cells and acts upon the Sertoli and peritubular cells of the seminiferous tubule and, via processes which are virtually unknown, drives spermatogenesis (Sharpe, 1986). Effect of season was not determined by environmental temperature only, but also by feeding system in each season. The extreme cold conditions during collection of winter season (December-February in Egypt) had an adverse effects on semen quality parameters of buffalo bulls.

The recorded poor semen characteristics in winter, in spite of higher thyroid activity, may resulted from the testicular displacement from the scrotum to the abdominal cavity and severe low environmental temperatures, leading to animal stressful (Zafar *et al*., 1988).

The non-significant differences in semen density, progressive motility and abnormality between winter and summer season may indicate that environmental factors, such as photoperiod, ambient temperature, relative humidity and type of feeds, had no obvious effect on testicular activities. This trend comes in agreement with the finding of Settergren and McEntee (1992), Hussain *et al* (2002), Mandal *et al*. (2003) and Koonjaenak *et al*. (2007). In this respect, photoperiod had no influence on testicular activity of buffaloes (Casao *et al*., 2010) and seasonal factors had no clear effect on the concentration of gonadotropins, FSH and LH (Brown *et al*., 1991).

Experiment 2 "Freezing ability of summer buffalo semen as affected by extender type"

Post-thawed sperm characteristics:

Data of sperm characteristics in post-thawed semen (Table 4) cleared that sperm characteristics enhanced ($P<0.05$) in semen extended with eMOLE in comparing with CEYm and control (CEY) extenders, in terms of significant ($P<0.05$) increases motility and livability along with decreasing abnormality, acrosome integrity and membrane integrity of spermatozoa. It is of interest to note that using eMOLE as an alternative for antibiotics in CEY (CEYm) slightly improved all sperm characteristics in post-thawed semen as compared to control extender (CEY) with Streptomycin sulphate and Penciline, but the differences were not significant. These results may indicate the save use of eMOLE as an extender or as antibiotics in CEY extender for freezing buffalo semen.

Motility, livability and abnormality have direct impacts on the fertilization process. Recently, there is an attention for evaluating integrity of sperm membrane as a fundamental indicator of the fertilization (Lodhi *et al*., 2008). Hypo osmotic swelling test could be a valuable and practical tool to know the functional capacity of fresh buffalo spermatozoa. It could be added to the routine analysis of semen samples for artificial insemination (Lodhi *et al*., 2008). Plasma membrane plays a significant role in pre-fertilization and fertilization processes (Azam *et al*., 1998). Thus, damage of plasma membrane and acrosome represents the major biological constraints for completion of acrosome reaction stage (Graham and Mocoe, 2005), and success of fertilization.

Table 4. Sperm characteristics in post-thawed buffalo semen extended by different types of extenders.

Extender type	Sperm characteristics (%)				
	Motility	Live sperm	Abnormality	ACI	MEI
CEY (control)	41.88± 2.10^{b}	43.88± 2.38^{b}	29.88± 1.26^{a}	28.00± 0.94^{a}	41.25± 1.29^{b}
CEYm	44.38± 1.48^{b}	45.75± 1.92^{b}	29.38± 0.78^{a}	27.63± 0.63^{a}	42.88± 1.01^{b}
eMOLE	55.00± 1.34^{a}	56.75± 1.10^{a}	22.75± 0.31^{b}	21.63± 0.50^{b}	57.88± 0.83^{a}

a and b: Different superscripts in the same column indicated significant differences at P<0.05.

ACI: Acrosome integrity. MEI: Membrane integrity.

The observed positive effect of eMOLE as an new extender in maintaining sperm characteristics during freezing process may be attributed to the effective role of eMOLE in scavenging the free radicals generation and subsequently decreasing the harmful effects of oxidative stress. Also, eMOLE reduces the antioxidant enzymes required to protect the cells from the free radicals (Arabshahi *et al*., 2007). The eMOLE contain carotenoids, alkaloids, and pro-anthocyanidins, which have ability to prevent activity impairment (Sadek., 2013). Also, MO contains vitamin C as pro- and anti-oxidant (Siddhuraju and Becker, 2003; Aslam *et al*., 2005). In an in vivo study of Syarifuddina *et al*. (2017), plasma testosterone concentrations increased and sperm motility improved in bulls treated with Moringa oleifera leaves.

Information on the anti-bacterial property of eMOLE in buffalo bull semen extender is rare (Sokunbi *et al*., 2015). The successful use of eMOLE as alternative for antibiotics in CEY extender in the current study agreed with (Dowidar *et al*., 2018) , who replaced antibiotics in Citrate extender by eMOLE in freezing buffalo bull semen, and this was mainly related to anti-bacterial and antioxidant properties of eMOLE (Sreelatha and Padma, 2010).

Enzyme activity in seminal plasma:

Activity of both AST and ALT in seminal plasma of cryopreserved semen was significantly ($P<0.05$) lower in eMOLE than in CEY, and insignificantly lower in CEYm than in CEY. Activity of LDH was significantly ($P<0.05$) the lowest in eMOLE, moderate in CEYm, and the highest in CEY.

Table 5. Activity of AST, ALT and LDH in seminal plasma of cryopreserved buffalo semen as affected by type of extender.

Extender type	Enzymatic activity (IU)		
	AST	ALT	LDH
CEY (control)	30.00±0.54^{a}	27.80±0.97^{a}	271.40±3.43^{a}
CEYm	28.80±0.49^{ab}	25.20±0.97^{a}	250.80±3.14^{b}
eMOLE	26.60±1.29^{b}	21.60±0.81^{b}	227.00±5.37^{c}

a and b: Different superscripts in the same column indicated significant differences at P<0.05.

AST: Asprtate transaminases. ALT: Alanine transaminases. LDH: Lactic dehydrogenase.

Several enzymes of the seminal plasma such as AST, ALT and LDH are considered as metabolic enzymes, providing the energy required to functional spermatozoa to

be motile, live and fertile. Activity of these enzymes are indicators of sperm plasma membrane integrity (Corteel, 1980). It is worthy noting that the observed reduction in enzyme activities is in association with increasing integrity of acrosome and plasma membrane of sperm cells during freezing process.

The obtained reduction in enzyme activity in seminal plasma of bull semen was reported by (Dowidar *et al*., 2018) in post-thawed semen of buffalo bulls. *Moringa oleifera* as antioxidants is known to suppress reactive oxygen species (ROS) formation and free radicals (Sofidiya *et al*., 2006; Ogbunugafor *et al*., 2011). Also, Abdou *et al*. (2012) reported that *Moringa oleifera* extract increased the production of antioxidants in the spermatozoa.

Pregnancy rate (%):

Insemination of buffalo cows with frozen semen extended with eMOLE significantly (P<0.05) increased pregnancy rate compared with semen extended with CEYm or CEY (83.33 vs. 66.66%, Figure, 1). These results are in matching with superiority of eMOLE as an extender in maintaining sperm metabolism, function and the best sperm characteristics and enzyme activity of semen extended with eMOLE.

Economic efficiency of different types of extenders:

Moringa is very impressive and amazing plant due to its potential benefits, it consider a gift of nature at very low price. It could easily and cheaply be cultivated and grown.

We could said that Moringa is the most inexpensive and credible alternative to any existing antibiotic, CEYm extender showed the cheapest cost half dollar (561 cent)/100 ml in comparing with CEY extender cost one dollar (1.085 cent)/100 ml. eMOLE as anew extender is economically efficient and reliable, cost 0.726 cent/100 ml comparing to conventional CEY extender cost one dollar (1.085 cent/100 ml)

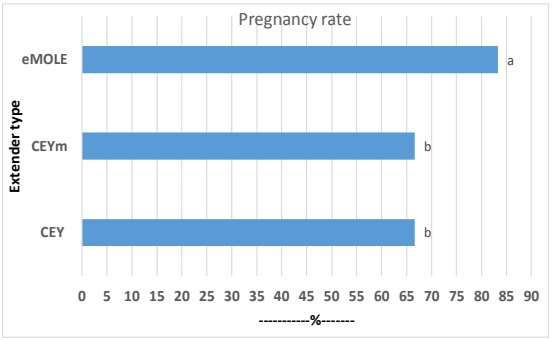

Figure 1. Effect of extender type on pregnancy rate of buffalo cows.

CONCLUSION

The recorded trend of season effect on sperm characteristics could be useful to meet the requirements of cryopreserved semen. Based on all previous obtained results, it is worthily nothing that, semen quality of buffalo bulls was better in summer than winter season in Egypt. Therefore, it can be recommended to collect and storage of the buffalo bull semen during summer for artificial insemination programs to enhance the pregnancy rates in buffaloes.

In addition, eMOLE is considered as a new promising extender for the dilution of cryopreserved buffalo semen and it could be used as an alternative for anti-biotics (4 ml) in CEY as a conventional semen extender.

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قدرات تجميد وتخصيب السائل المنوي لطلائق الجاموس المصري في الصيف باستخدام مستخلص المورينجا كمضاد حيوي أو كمخفف واعد جديد

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جمع السائل المنوي هو أساس العمل لمختلف منظمات التلقيح الاصطناعي، وبالتالي فإن إدارة عملية الجمع بأكملها أمر بالغ الأهمية. لذلك، فإن الاتجاه الآن هو تصميم الإجراءات أو صقل المنهجيات من أجل زيادة إنتاج السائل المنوي ذات النوعية الجيدة إلى أقصى حد دون التخلص من القذف ذات النوعية الرديئة. يهدف هذا العمل إلى دراسة تأثير فصلي (الشتاء - الصيف) على خصائص السائل المنوي للجاموس ، وتقييم قدرة التجميد والتخصيب للسائل المنوي للجاموس والتي تم جمعها في الصيف وتخفيفها باستخدام المستخلص الايثانولي للاوراق المورينجا كمخفف وكمضاد حيوي طبيعي ومضاد أكسدة في المخفف. تم جمع السائل المنوي مرتين في الأسبوع عن طريق المهبل الاصطناعي من أربعة طلائق جاموسي ناضجة 400 – 450 كجم وزن حي وعمرمن 4 - 5 سنوات خلال فصلي (الشتاء – الصيف) لمدة 10 أسابيع في الشتاء (ديسمبر - فبراير) وفي الصيف (يوليو- أغسطس). تم تقييم بعض خصائص السائل المنوي للقذفات خلال كلا الموسمين (التجربة الأولى). في التجربة الثانية ، تم تخفيف القذفات ≤70٪ فقط كحركة جماعية للحيوانات المنوية بواسطة مخفف السترات + صفار البيض ككنترول(CEY) والسترات مضاف الية eMOLE بديلا عن المضادات الحيوية(CEYm)، ومخفف eMOLE وتم حفظهم بالتجميد في النيتروجين السائل (-196 درجة مئوية) تم تقييم السائل المنوي بعد الاسالة خصائصيا وكيميائيا، ومعدل الحمل كما تمت تجربة الخصوبة. أوضحت النتائج أن حجم القذفة والنسب المئوية للحيوانات المنوية الحية وسلامة الأكروسوم وسلامة الغشاء زادت معنويا ($P<0.05$) في فصل الصيف مقارنة بفصل الشتاء. ومع ذلك، فإن تأثير الموسم على كثافة السائل المنوي، والحركة التقدمية والحيوانات المنوية الشاذة لم تكن معنويا. تحسنت خصائص السائل المنوي المخفف بـ eMOLE معنويا(الحركة التقدمية، ونسبة الحي، والشذواذ، وسلامة الاكروسوم) بعد الاسالة. وأيضا النشاط الإنزيمي(AST, ALT, LDH) خفضت إلى الحد الأدنى من المستويات في بلازما السائل المنوي، لم يكن لاستبدال المضادات الحيوية في مخفف CEY بـ eMOLE (4 مل / 100 مل) أي تأثير كبير على جميع خصائص الحيوانات المنوية ونشاط الإنزيم. أظهر السائل المنوي المخفف باستخدام مستخلص المورينجا الايثانولي معدل خصوبة أعلى($P <0.05$) معنويا (83.3 %) مقارنةً بالمخفف CEY او CEYm (66.6 %). في الختام ، يمكن جمع السائل المنوي للجاموس خلال أشهر الصيف (يوليو - أغسطس) في مصر واستخامها في التلقيح الاصطناعي بالتعاون مع جمع السائل المنوي الجيد في فصل الشتاء. من الناحية الاقتصادية ، يُعتبرمستخلص المورينجا الايثانولي مخفف جديدًا وواعدًا وبديلًا أيضا عن المضادات الحيوية ومضادات الأكسدة الفعالة في مخففات السائل المنوي للجاموس المصري وحفظها بالتجميد خلال فصل الصيف.