QUALITY AND FERTILITY RATE OF FROZEN BUFFALO SEMEN AS AFFECTED BY ADDITION OF EXTRACT OF MORINGA LEAVES AND VARIOUS THAWING RATES

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SUMMARY

Five sexually mature Egyptian buffalo bulls were used as semen donors for 15 weeks in this investigation to evaluate the impact of adding various concentrations of methanolic extract of moringa leaves (MEML) to the freezing skim camel milk (SCM) extender and thawing regimes (TR) on post-thaw quality, freezability, metabolic activity and fertility rate of buffalo spermatozoa. The selected ejaculates with ≥ 95% sperm normality and ≥ 90% initial sperm motility were pooled and extended in SCM extender and separated into 4 equal portions including control without any administration of MEML, and three concentrations of MEML (3,9 and 18%, respectively). Extended semen without or with three concentrations of MEML were packaged in straws (0.25 mI), equilibrated at 5°C for 6 hours and freezed in liquid nitrogen container for storage at -196°C for 60 days. Thereafter, the cryopreserved straws were thawed at 65°C for 10 seconds, 50°C for 20 seconds and 35°C for 30 seconds in water bath for estimation post-thaw quality, freezability, metabolic activity and fertility of buffalo spermatozoa. The results showed that technical parameters of buffalo spermatozoa, levels of total antioxidants, lactic dehydrogenase enzyme and malondialdehyde in post-thaw seminal plasma of frozen-semen of buffalo bulls were better (P<0.05) with all levels of MEML as compared to that of control (free from MEML). The ideal results (P<0.05) were recorded for diluted semen that enriched with 9% MEML. Also, the optimal results (P<0.05) of post-thaw semen characteristics, metabolic activity and fertilizing efficiency of buffalo spermatozoa were assessed when thawing was done for cryopreserved straws at 65°C for 10 seconds. Conclusively, it is recommended to extend and freeze buffalo semen with skim camel milk extender fortified with MEML at a level of 9% for artificial insemination programs to enhance post-thaw semen quality, freezability and metabolic activity of spermatozoa, and subsequent increase conception rate of inseminated buffaloes, particularly when thawing was done for cryopreserved straws at 65°C for 10 seconds.

Keywords: *buffalo semen, moringa oleifera, thawing, cryopreservation, conception rate.*

INTRODUCTION

The Egyptian buffaloes is considered as one of the genetic resources of native Egyptian livestock that must be improved, protected and preserved (El-Nagar *et al.*, 2021). The principal problem to exploitation of frozen semen is freezing and/or thawing procedures of buffalo spermatozoa, finally leading to highly reduce post-thaw quality and fertilizability potential of spermatozoa (Pertrghella *et al.*, 2017 and Chavda *et al.*, 2022). Although, cryopreservation of semen is an important section of artificial insemination programs, it is oftentimes associated with a marked reduction in functionality and characteristics of buffalo spermatozoa as well as increasing peroxidative injury as compared to cattle spermatozoa (Andrabi, 2009 and Lone *et al.*, 2018). Also, buffalo spermatozoa are very sensitive to reactive oxygen species (ROS) because of less activities of endogenous antioxidant enzymes and high content of polyunsaturated fatty acids of the phospholipids in plasma membranes (Kadirvel *et al.*, 2009 and Kobaide, 2016). Moreover, the higher production of malondialdehyde (MDA) in buffalo spermatozoa through lipid peroxide (LP) could be due to more unsaturated fatty acids in the plasma membranes than other species so that spermatozoa become more susceptible to lipid peroxidation (Chavda *et al.*, 2022). Semen preservation increases peroxidation of spermatozoa, so that an adequate antioxidant protection

can be directly supplemented to the medium (Wahjuningsih *et al.,* 2019 and Wafa *et al.,* 2021) or by dietary administration (El-Nagar *et al.,* 2021 and Gabr *et al.,* 2023). Function of the semen extender is to provide the spermatozoa with nutrients, prevent the spermatozoa from temperature-related injury, control bacterial contamination, and maintain ideal conditions for the spermatozoa (Raheja *et al.,* 2018 and Wafa *et al.,* 2021). In order to maximize the quality of buffalo semen-extender to achieve an optimal post-thawing semen measurement and subsequently conception rate of inseminated buffaloes, it is important to investigate the influence of administration of the extender with natural source of antioxidants.

Over the years, there are several natural antioxidants additives have been used to improve post-thawing sperm characteristics, fertilizability potential of spermatozoa and subsequent pregnancy rate. Among those additives is moringa leaves which has a high content of natural antioxidants such as carotenoids (especially carotene and lutein), flavonoids and alkaloids (Oparinde and Atiba, 2014) and rich in essential amino acids (lysine, tryptophan, methionine and cysteine) and vitamin B, C and A with a high content of proteins (Mendieta-Araica *et al.,* 2011). Also, previous publications observed the potent antioxidants of extract of moringa leaves, that allow to be supplementing it in extender of ram (El-Harairy *et al.,* 2016), goat (Wahjuningsih *et al.,* 2019), buffalo (Dowidar *et al.,* 2018 and El-Nagar *et al.,* 2019) and bovine (Hammed *et al.,* 2019). Beside the antioxidant properties of moringa leaves, it can act as antibacterial, antitumor and antifungal (Sokunbi *et al.,* 2015 and Okediran *et al.,* 2017). Supplementing methanolic extract of moringa leaves (MEML) to diluted semen as a source of antioxidants and antibiotics could improve post-thawing characteristics and freezability of buffalo spermatozoa and enhance pregnancy rate of inseminated buffaloes (Dowider *et al.,* 2018 and El-Nagar *et al.,*2019). In addition, extract of moringa leaves possesses a powerful scavenging role for free radicals that arises during lipid peroxidation (Okediran *et al.,* 2017).

Thawing regime is just as important as the freezing proceduce in terms of its effect upon survival rate and quality of spermatozoa (Correa *et al.,* 1996 and Nur *et al.,* 2003). Previous publications have illustrated that while temperature degree more than 35°C can improve motility of spermatozoa, the duration of thawing must be shortened and carefully timed (El-Shennawy, 2013). Also, several investigations were carried out to define the best thawing regime of frozen semen in straws, in order to optimize post-thaw quality and fertilizing ability of spermatozoa (Nur *et al.,* 2003; Zeidan *et al.,* 2004 and Gabr *et al.,* 2023). The general theory is that fast thawing proceduce for frozen semen in straw is advantageous to reduce or avoid injury through re-warming (Vishwanath and Shannon, 2000). The principal problem in slow thawing regime of frozen semen is the osmotic change due to water ingress through the process, as this is more injurious to spermatozoa compared to egress of water through freezing (Marai *et al.,* 1998 and Zeidan *et al.,* 2004). In this respect, Curry and Watson (1994) and Kobadie (2016) mentioned that when frozen straws were thawed ideally, the formation of intracellular ice-crystal may be prevented. Recently, Gabr (2023) pointed out that rapid thawing procedure of frozen semen in straws is more suitable for improving survival, viability, and acrosomal and plasma membrane integrities of bull spermatozoa as well as increasing pregnancy percentage of inseminated cows compared to moderate or slow thawing procedure. The evident advantage of rapid thawing procedure for frozen straws could be explained upon the basis that each straws makes a certain special type of plastic coat around the semen which required a high thawing temperature (Almquist and Wiggin, 1973 and Marai *et al.,* 1998). The main goal of this investigation was to monitor the influence of administration of semen buffalo-extender with various concentrations of methanolic extract moringa leave (MEML) and thawing regimes upon post-thaw semen quality, freezability, metabolic activity and fertilizing ability of spermatozoa.

MATERIALS AND METHODS

Bulls and semen collection:

Five mature and healthy Egyptian buffalo bulls, aged between 3 to 4 years old and weighed 430 to 520 kg were used as semen donors. Bulls were housed individually under identical managerical and nutritional conditions at El-Gemmizah Animal production Experimental Station, Animal Production Research Institute (APRI), Agricultural Research Center, Egypt. Bulls were fed separately on diet composed of rice straw (RS), concentrate feed mixture (CFM) and clover hay (CH) according to APRI (2002). The experimental bulls had free choice to drink clean water through the day time. Table (1) display the chemical analysis of feed ingredients RS, CFM and CH.

Throughout semen collection of 15 weeks, semen ejaculates were collected in the morning hours once weekly from the experimental bulls by a sterile artificial vagina (IMV, France). Just post semen collection, tubes containing semen ejaculates were placed in a water bath at 37°C and transported to the laboratory for examination and cryopreservation of semen.

Table (1): Chemical analysis of feed ingredients.

Item	Chemical analysis (% on DM basis)						
	DM	OM	EE	CP	NFE	CF	ASH
Rice straw	89.95	83.81	1.40	3.40	44.11	34.90	16.19
Concentrate feed mixture	92.06	92.10	4.15	16.33	55.40	16.22	7.90
Clover hay	91.27	89.37	1.31	11.69	42.89	33.48	10.63

Extension, administration and cryopreservation of semen:

The selected ejaculates with ≥ 95% sperm normality and ≥ 90% initial sperm motility were pooled and immediately extended with (SCM) extender at 1: 8 extension rate. Composition of SCM extender fortified with various concentrations of MEML are shown in (Table 2). Extended semen was divided into 4 equal portions. The first portion was left without any administration of MEML and considered as a control. While the other three portions (2,3 and 4) were administrated with various levels of MEML (3,9 and 18 %, respectively). MEML was prepared according to (Sokunbi *et al.,* 2015). Soon post final extension of semen that incorporated with various concentrations of MEML, the extended semen portions with or without various concentrations of MEML were aspirated in straws (0.25 ml), sealed with polyvinyl alcohol powder, then straws were left at 5°C for 6 hr as equilibration time and all straws were freezed in liquid nitrogen container at -196°C (Salisbury *et al.,* 1978) for preservation. Frozen-straws preserved for 60 days were thawed at 65°C for 10 seconds, 50°C for 20 seconds and 35°C for 30 seconds in water bath for estimation of post thaw quality, freezability, metabolic activity and fertility of buffalo spermatozoa.

Table (2): Composition of skim camel milk extender fortified with various concentrations of methanolic extract of moringa leaves.

Components	Semen extender media			
	G1	G2	G3	G4
Sodium citrate dehydrate, g	2.90	2.90	2.90	2.90
Citric acid anhydrous, g	0.04	0.04	0.04	0.04
Skim camel milk, ml	10	10	10	10
Egg yolk, ml	20	20	20	20
Glycerol, ml	7	7	7	7
Penicillin, IU/ml	500	500	500	500
Streptomycin, mg/ml	500	500	500	500
Distilled water, ml up to	100	100	100	100
Methanolic extract of moringa leaves levels, ml	0	3	9	18

Evaluation of post-thawed semen:

The preserved seminal plasma samples were thawed and assayed for lactic dehydrogenase activity (Howell *et al.,* 1979) and total antioxidant activity (Koracevic *et al.,* 2001), by commercial kits (Salucea, Netherlands) using spectrophotometer (Jenway, 6405UV/Vis, England). Also, malondialdehyde concentration was determined according to (Richard *et al.,* 1992).

Fertility rate:

Forty healthy buffaloes with normal heat and estrous were randomly allotted to four groups (one control and three treatments, each including 10 cows), and were artificially inseminated (AI) by semen diluted with SCM extender fortified with (0,3,9 and 18% MEML, respectively), and thawed at 65°C for 10 seconds (the ideal thawing rate). For each cow, two inseminations were done in the evening and morning of the same day. Conception rates for the inseminations buffaloes were computed on the basis of pregnancy diagnosis via rectal examination post 60 days from the day of insemination.

Statistical analysis:

All data were analyzed using the SPSS software (IBM, SPSS Statistics, Version 22, USA). The data were expressed as mean ± standard error of means (SEM). Two-way ANOVA was used to compare the influence of MEML supplementation and thawing regimes on post-thaw semen characteristics and enzymatic activity Duncan Multiple Range test (Duncan, 1955) was used to differentiate between significant means at $P < 0.05$. The percentage values were transferred into arc-sin prior being analysis and computed as means. The conception rates were analyzed using Chi-square test.

RESULTS AND DISCUSSION

Post-thaw sperm characteristic:

Post-thaw viability, normality, motility, membrane integrity and acrosomal damage percentages of buffalo spermatozoa were better ($P<0.05$) with all levels of MEML as compared to the control (free from MEML), the best values ($P<0.05$) of post-thaw characteristics of spermatozoa were recorded when diluted semen enriched with 9% MEML. Such results indicated a powerful protective impact of MEML on functions and characteristics of cryopreserved buffalo spermatozoa, that was level dependent, and it was more pronounced in semen fortified with 9% MEML than those fortified with 3 or 18% MEML (Table 3). The results are in accordance with those of (Wahjuningsih *et al.,* 2019) in goat, (Dowidar *et al.,* 2018 and El-Nagar *et al.,* 2019) in buffalo and (Hammad *et al.,* 2019) in bull spermatozoa, who mentioned that in vitro administration of semen extenders with MEML resulted in pronounced improving of post-thaw survival rate, integrity of cell membrane and viability of spermatozoa, besides remarkable reduction in post-thaw abnormalities and acrosomol injury of spermatozoa. The protective impacts of MEML upon functions and characteristics of spermatozoa may be attributed to powerful antioxidants and antiradicals properties, which plays a major role in the protection of spermatozoa against peroxidative injury of ROS (Oparinde and Atiba, 2014 and Sokunbi *et al.,* 2015). Also, El-Nagar *et al.* (2019) reported that antioxidant content of MEML was able to maintain the acrosome membrane integrities as well as quality of buffalo spermatozoa during cryopreservation. Moreover, Wafa *et al.,* 2017 concluded that enriching buffalo bulls diet by natural source of antioxidants like moringa leaves led to improve semen production and optimize post-thaw quality of spermatozoa.

In relation to thawing procedures for frozen semen, thawed frozen semen at 65°C for 10 seconds improved post-thawed survival and quality of spermatozoa as reflected by viability, normality, motility, membrane integrity and acrosomal damage percentages of buffalo spermatozoa as compared to 50°C for 20 seconds or 35°C for 30 seconds (Table 3). Also, no significant differences were observed in characteristics of spermatozoa between frozen semen thawed at 50°C for 20 seconds and 35°C for 30 seconds. In general, the best ($P<0.05$) post-thaw survival rate and characteristics of spermatozoa were detected in semen extended with SCM extender fortified with 9% MEML, and thawed at 65°C for 10 seconds (rapid thawing regime). Our finding was strongly supported by those of Gabr (2009) and kobaide (2016) in buffalo and Marai et al. (1998) and Zeidan et al. (2004) in bull spermatozoa who indicated that rapid thawing regime of frozen semen was more suitable for improving post-thaw measurements, membrane and acrosomal integrities and metabolic activity of spermatozoa as compared to slow or moderate thawing regimes. Also, Marai et al. (1998) and Zeidan et al. (2004) found that the basic problem in slow thawing regime of spermatozoa is the osmotic change due to water ingress through the process, since it was considered more injurious to spermatozoa as compared to egress of water through freezing. In this respect, the evident advantage of rapid thawing regime for frozen straws was explained on the basis that each straw makes a certain special type of plastic coat around the semen which requires high thawing temperature (Almquist and Wiggin, 1973 and Marai *et al.,* 1998). Also, previous publications have illustrated that while temperature degree more than 35°C result in improve motility of spermatozoa, it should be pointed out that the duration of thawing must be shortened and carefully timed (El-Shennawy, 2013). Post-thawing total morphological and defected acrosome rates as well as motility and plasma membrane integrity of bull spermatozoa were affected by thawing regimes, being optimal when frozen semen was thawed at 70° for 5 seconds (Nur *et al.,* 2003). Recently, Gabr et al. (2023) pointed out that rapid thawing procedure of frozen semen in straws is more suitable for improving survival rate, viability, and acrosomal and plasma membrane integrities of bull spermatozoa compared with moderate or slow thawing procedures

Table (3): Post-thaw buffalo spermatozoa characteristics as affected by various concentrations of MEML and thawing regimes (TR).

Sperm characteristics (%)	MEML levels (%)	Thawing regimes (°C/second)			Overall means
		65°C/10 sec	50°C/20 sec	35°C/30 sec	
Viability (%)	0	48.34 ± 3.70	38.51 ± 3.15	35.31 ± 3.90	40.72^{c} ± 3.01
	3	63.11 ± 1.18	50.96 ± 2.17	48.35 ± 1.15	54.14^{b} ± 1.40
	9	71.05 ± 0.63	62.73 ± 0.91	58.11 ± 1.01	63.96^{a} ± 0.87
	18	60.57 ± 0.93	53.88 ± 1.83	50.77 ± 1.35	55.07^{b} ± 1.53
Overall means		60.75^{A}±0.88	51.52^{B}±1.19	48.14^{B}±1.02	
Normality (%)	0	63.11 ± 0.94	58.99 ± 2.21	54.75 ± 2.80	58.95^{c} ± 1.98
	3	75.97 ± 0.60	63.88 ± 0.81	60.19 ± 1.06	66.62^{b} ± 0.71
	9	88.33 ± 0.74	80.77 ± 0.96	78.93 ± 1.03	82.68^{a} ± 0.65
	18	70.88 ± 0.78	60.51 ± 0.88	58.38 ± 0.95	63.26^{b} ± 0.81
Overall means		74.57^{A}±0.72	66.04^{B}±1.03	63.06^{B}±0.98	
Motility (%)	0	44.37 ± 3.01	29.27 ± 4.20	26.13 ± 4.70	33.26^{c} ± 3.25
	3	48.72 ± 2.85	38.50 ± 1.95	31.17 ± 1.40	39.46^{b} ± 1.91
	9	69.95 ± 0.90	48.37 ± 1.03	41.35 ± 1.15	53.22^{a} ± 0.73
	18	50.35 ± 1.35	43.34 ± 1.25	38.21 ± 1.40	43.97^{b} ± 1.51
Overall means		53.33^{A}±0.95	39.87^{B}±2.25	34.22^{B}±2.83	
Membrane integrity (%)	0	45.30 ± 1.31	35.19 ± 1.70	38.37 ± 1.50	39.62^{c} ± 1.05
	3	56.33 ± 0.89	43.17 ± 0.54	40.73 ± 0.40	46.74^{b} ± 0.61
	9	68.98 ± 0.81	58.98 ± 0.91	53.11 ± 1.15	60.36^{a} ± 0.73
	18	51.93 ± 0.87	39.33 ± 0.61	35.98 ± 0.71	42.41^{b} ± 0.87
Overall means		55.64^{A}±0.77	44.17^{B}±0.99	42.05^{B}±0.66	
Acrosmal damage (%)	0	30.27 ± 1.45	45.31 ± 2.31	48.93 ± 2.30	41.50^{a} ± 1.99
	3	19.56 ± 0.53	25.27 ± 0.83	27.37 ± 0.61	24.07^{b} ± 0.76
	9	13.21 ± 0.25	18.40 ± 0.78	21.31 ± 0.81	17.64^{c} ± 0.35
	18	22.81 ± 0.28	28.55 ± 0.68	30.98 ± 0.86	27.45^{b} ± 0.66
Overall means		21.46^{B}±0.27	29.38^{A}±0.66	32.15^{A}±0.78	

Means bearing various letters within the same classification, differ significantly at (P<0.05)

Antioxidants and lipid peroxidation state in seminal plasma:

Concentrations of total antioxidants, lactic dehydrogenase enzyme and malondialdehyde in seminal plasma of post-thawed buffalo semen were better (P<0.05) with all concentrations of MEML than that of the control, and the ideal results were recorded with a concentration of 9% MEML (Table 4). These findings proofed beneficial influence of all MEML on reducing concentrations of lactic dehydrogenase enzyme and malondialdehyde, while increasing concentration of total antioxidants in seminal plasma of post-thawed buffalo semen, that was concentration dependent, and it was more pronounced in extended semen fortified with 9% MEML than fortified with 3 or 18% MEML. These findings go hand in hand with the results obtained by Dowider et al. (2018) and Hammed et al. (2019) who noticed the positive influence of MEML as a natural source of antioxidants, which are known to decrease formation of reactive oxygen species and free radicals that arise during lipid peroxidation. El-Nagar et al. (2019) showed the beneficial impact of MEML in removing free radicals, inhibiting oxidases and activities of antioxidant enzymes. Wafa et al. (2021) stated that semen with perfect quality can be characterized by a decrease in the activity of metabolic enzymes and increase in concentration of antioxidant enzymes.

In relation to thawing procedures for frozen semen, frozen straws thawed at 65°C for 10 seconds reduced (P<0.05) levels of lactic dehydrogenase enzyme and malondialdehyde, and increased (P<0.05) concentration of total antioxidants in plasma of post-thawed buffalo semen as compared to 50°C for 20 seconds or 35°C for 30 seconds (Table 4). Also, no significant differences were observed in the concentrations of lactic dehydrogenase enzyme, malondialdehyde and total antioxidants in seminal plasma between thawing frozen semen at 50°C for 20 seconds and 35°C for 30 seconds. Our results agreed with those recorded by (Marai *et al.,* 1998; Nur *et al.,*2003 and Zeidan *et al.,* 2004). Also, Gabr, 2009 found that the ideal thawing procedure with antioxidant supplementation to freezing extender of buffalo spermatozoa was 55°C/15 sec. (rapid thawing procedures). Recently, Gabr *et al.,* 2023 found that concentrations of hyaluronidase enzyme and malondialdehyde were lower (P<0.01), and total antioxidants capacity was higher (P<0.01) in seminal plasma of post-thaw bull semen when straws were

thawed at 55°C/15 sec (rapid thawing protocol) as compared to thawing 15°C/60 sec or at 35°C/30 sec. On the other hand, Pontbriand et al. 1989 did not observe positive impact for thawing procedures upon post-thawing characteristics or activity of enzymes in seminal plasma of ram post-thawing at 60°C/8sec or 37°C/20 sec using 0.5 ml straws.

Table (4): Total antioxidants, lactic dehydrogenase enzyme and malondialdehyde in post-thaw seminal plasma of buffalo semen as affected by various concentrations of MEML and thawing regimes.

Item	MEML levels (%)	Thawing regimes (°C/second)			Overall means
		65°C/10 sec	50°C/20 sec	35°C/30 sec	
Total antioxidants (mmol/l)	0	12.14 ± 1.90	7.30 ± 0.90	6.67 ± 0.05	8.70^{c} ± 0.99
	3	19.99 ± 0.17	13.25 ± 0.78	11.27 ± 0.77	14.84^{b} ± 0.50
	9	25.17 ± 0.03	19.71 ± 0.01	17.53 ± 0.03	20.80^{a} ± 0.02
	18	17.33 ± 0.11	12.72 ± 0.39	10.97 ± 0.61	13.67^{b} ± 0.30
Overall means		18.67^{A} ± 0.66	13.25^{B} ± 0.37	11.61^{B} ± 0.41	
Lactic dehydrogenase (IU/l)	0	230.31 ± 5.31	290.47 ± 6.34	315.71 ± 7.31	278.81^{a} ± 6.43
	3	180.83 ± 4.13	254.31 ± 4.62	265.31 ± 6.72	233.48^{b} ± 5.11
	9	125.85 ± 3.35	190.43 ± 4.37	231.33 ± 5.11	182.52^{c} ± 3.33
	18	191.50 ± 5.95	271.72 ± 3.76	280.22 ± 4.14	248.03^{b} ± 4.99
Overall means		182.05^{B} ± 4.31	251.73^{A} ± 5.41	273.44^{A} ± 5.91	
Malondialdehyde (µM)	0	25.11 ± 1.11	38.30 ± 2.05	41.50 ± 2.23	34.88^{a} ± 1.50
	3	15.33 ± 0.53	24.05 ± 0.61	27.31 ± 0.90	22.23^{b} ± 0.61
	9	11.02 ± 0.41	17.05 ± 0.70	20.31 ± 0.81	16.15^{c} ± 0.45
	18	18.11 ± 0.76	28.77 ± 0.83	31.81 ± 0.95	26.21^{b} ± 0.35
Overall means		17.39^{B} ± 0.51	27.01^{A} ± 0.61	30.23^{A} ± 0.51	

Means bearing various letters within the same classification, differ significantly at (P<0.05)

Fertility rate:

Table 5. display the percentages of pregnancy of buffaloes inseminated by frozen-semen thawed at 65°C for 10 seconds with various levels of MEML. The diluted semen enriched with 9% MEML had greatly higher (90.90%) as compared to semen fortified with 3 or 18% MEML (72.72 for each) and semen without administration of MEML (54.55%, the control semen). Generally, all concentrations of MEML had pronounced influence on pregnancy percent. These findings are compatible with those of (Dowidar *et al.,* 2018 and El-Nagar *et al.,* 2019) who mentioned that in-vitro administration of buffalo semen extender with MEML enhanced post-thaw semen parameters and its fertilizability, consequently improved pregnancy percent. Antioxidants properties of MEML may decreased the viscosity of semen that is important for enhancing the fertilizing ability of spermatozoa (Hammad *et al.,* 2019). Post-thaw fertilizing ability of spermatozoa is also highly affected by thawing technique such as temperature and/or duration (Senger, 1980 and Nur *et al.,* 2003). In this respect, rapid thawing procedure of frozen-semen is more suitable for enhancing survival and fertilizability potential of spermatozoa compared with moderate or slow procedures (Marai *et al.,* 1998; Zeidan *et al.,* 2004 and Gabr *et al.,* 2023).

Table (5): Pregnancy (%) for buffaloes inseminated by frozen semen fortified with various concentrations of MEML and thawed at 65°C for 10 seconds.

Item	MEML concentrations			
	0	3	9	18
Number of inseminated buffaloes	11	11	11	11
Number of conceived buffaloes	6	8	10	8
Pregnancy %	54.55^{c}	72.73^{b}	90.90^{a}	72.73^{b}

Means bearing various letters within the same classification, differ significantly at (P<0.05)

CONCLUSION

Conclusively, it is recommended to extend and freeze buffalo semen with skim camel milk extender fortified with MEML at a level of 9% for artificial insemination programs to enhance post-thaw semen quality, freezability and metabolic activity of spermatozoa, and subsequent increase conception rate of inseminated buffaloes, particularly when thawing was done for cryopreserved straws at 65°C for 10 seconds.

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جودة ومعدل خصوبة السائل المنوي المجمد من الطلائق الجاموسي وعلاقتها بإضافة مستخلص أوراق المورنجا ومعدلات الإسالة المختلفة

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في هذه الدراسة تم تجميع قذفات السائل المنوي من 5 طلائق جاموسي ناضجة جنسياً بواسطة المهبل الصناعي بمعدل مرة أسبوعياً لمدة 15 أسبوع لتقييم تأثير إضافة تركيزات مختلفة من المستخلص الميثانولي لأوراق المورنجا(MEML) إلى مخفف السائل المنوي المجمد مع معدلات الإسالة المختلفة علي جودة السائل المنوي والنشاط الأيضي وخصوبة الحيوانات المنوية لطلائق الجاموس المصري بعد الإسالة. تم خلط قذفات السائل المنوي ذات النسبة المئوية للحيوانات المنوية الطبيعية أكبر من أو تساوي 95% و ذات الحركة أكبر من أو تساوي 90% فقط في كل مرة جمع، ثم خففت بمخفف لبن الأبل الفرز و قسمت إلي أربع أجزاء هي المقارنه (بدون إضافة) وثلاث تركيزات من(MEML 3) ، 9 ، 18% .(ثم تم عمل فترة موازنة لقصيبات السائل المنوي علي درجة حرارة 5°م لمدة 6 ساعات ثم حفظت في النيتروجين السائل (-196°م) لمدة 60 يوم. وفي نهاية فترة الحفظ تم إسالة قصيبات السائل المنوي المجمد علي معدلات إسالة مختلفة (سريع: 65°م لمدة 10 ثوان ، متوسط: 50°م لمدة 20 ثانية ، بطئ: 35°م لمدة 30 ثانية) و ذلك لتقييم جودة السائل المنوي و قدرة الحيوانات المنوية علي تحمل التجميد (القابلية للتجميد) و النشاط الأيضي و خصوبة الحيوانات المنوية بعد التجميد والإسالة .

أظهرت الدراسة تحسناً معنوياً ملحوظاً (علي مستوي 5%) في النسبة المئوية لحيوية الحيوانات المنوية والحيوانات المنوية الطبيعية وذات الغشاء الخلوي السليم و الحيوانات المنوية ذات الأكروسوم غير السليم بعد الإسالة بالإضافة إلي تركيز مضادات الأكسدة وإنزيم اللاكتيك ديهيدروجينز و المالون دي-الدهيد في بلازما السائل المنوي بعد الإسالة و ذلك مع كل تركيزات MEML مقارنة بدون إضافة المستخلص الميثانولي (الضابطة). وكانت أفضل النتائج عند إضافة MEML بتركيز 9%. أيضا قدرت أفضل قيم لخصائص السائل المنوي و النشاط الأيضي و الكفاءة الإخصابية للحيوانات المنوية المجمدة عند إسالتها علي درجة حرارة 65°م لمدة 10 ثوان ، و بناء عليه نوصي من خلال نتائج هذه الدراسة بتخفيف و تجميد السائل المنوي من الطلائق الجاموسي بمخفف لبن الأبل الفرز مع إضافة المستخلص الميثانولي لأوراق المورينجا بتركيز 9% عند إجراء برامج التلقيح الاصطناعي لتعزيز جودة السائل المنوي بعد الإسالة وقابليته للتجميد و النشاط الأيضي للحيوانات المنوية و بالتالي زيادة نسبة الإخصاب في الجاموس المصري خاصة بعد الإسالة علي درجة حرارة 65°م لمدة 10 ثوان.