J.Animal and Poultry Prod., Mansoura Univ., Vol. 7 (7): 225 - 231, 2016

Effect of Leptin and Melatonin as Protective Additives to Tris Extender on Frozen Semen Quality of Buffalo Bulls

Abdel-Khalek, A. E.*; H. A. El-Nagar and Omima M. Ibrahim****

*** Animal Production Department, Faculty of Agriculture, Mansoura University. Egypt.**

****Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt.**

ABSTRACT

This study was conducted at Animal Production Research Station, El-Gemmezah, belonging to Animal Production Research Institute, Egypt, in cooperation with Department of Animal Production, Faculty of Agriculture, Mansoura university. Aim of the current study was to evaluate the possible effects of leptin and melatonin as a protective additive in Tris-extender on frozen semen quality of buffalo bulls. Five sexually mature Egyptian buffalo bulls (aged 4 to 5 years) with an average live body weight of (450-500 kg). All bulls were fed on the same diet. Semen was collected, twice a week for 4 wk (40 ejaculates for each bulls), using artificial vagina. The collected ejaculates (mass motility of more than 70%) were pooled, evaluation, divided into 7 replicates and frozen. Semen was extended in Tris- egg yolk extender as unsupplemented (T1), or supplemented with leptin (10, 20 and 50 ng/ml in T2, T3 and T4, or melatonin (10^{-3}M, 10^{-6}M and 10^{-9}M in T5, T6 and T7), respectively. Semen was diluted , equilibrated at 5^0C for 4 h and frozen in liquid nitrogen. After storage for 4 weeks, frozen straws were thawed at 37^0C for 30 sec. Semen was evaluated in post-dilution, post-equilibrated and post-thawed semen. Various sperm parameters including percentage of individual motility (IM), dead sperm (DS) and sperm abnormality (SA) were determined. In the seminal plasma of thawed semen, activity of AST, ALT and LDH was measured. Total of 12 buffalo cows in heat were inseminated by semen extended with T1, T3 and T5 to determine conception rate (CR). Pregnancy rate was measured by rectal palpation on day 50 after (AI). The obtained results show that IM and SA percentages improved ($P<0.05$) in T3 and T5. Percentage of DS improved ($P<0.05$) in all treatments as compared to control. T3 showed the best ($P<0.05$) results concerning sperm characteristics studied in post-diluted as compared to other additives and T1. IM, DS and SA percentages showed the best ($P<0.05$) results in post-equilibrated semen (T3), followed T5 in comparison with other additives and control. IM, DS and SA improved ($P<0.05$) in post-thawed semen diluted (T3) or T5, being better with T3 more than with T5. Enzyme activity of AST, ALT and LDH reduced ($P<0.05$) in seminal plasma of post-thawed semen diluted with all additives as compared to control, being the lowest ($P<0.05$) in T3. Buffalo cows inseminated with semen (T3) showed higher ($P<0.05$) CR (91.6%) than T5 (75.0%) or control (T1, 66.6%). In conclusion, supplementation of 20 ng/ml leptin and 10^{-3} M melatonin to the freezing semen extender improved semen quality and reduce cryodamage of the buffalo bull spermatozoa.

Keywords: Buffalo, semen, leptin, melatonin, freezing process, enzyme.

INTRODUCTION

Buffalo population in the world is continuously increasing and estimated to be more than 170 million head ((FAO) 2004). The domestic buffalo was a distinct species within the bovidae family, where buffaloes played a prominent role in rural livestock production providing the milk, meat, leather and work draft force (Andrabi, 2009 and kumar *et al* ., 2011).

Semen cryopreservation offers many advantages to the livestock industry, the production potential of livestock can be increased by genetic improvement using one of the modern ways of breed improvement, e.g. artificial insemination (AI) (Bucak *et al*., 2009). Using AI potentially has an important role in livestock breeding. It makes the dissemination of genetic material from a small number of superior sires to a large number of females possible, but the success of an AI program depends on the proper management of semen collection, storage and use (Leboeuf *et al*., 2000). During cryopreservation process to keep the cell sperm alive, toxicity of cryoprotectants and osmotic stress reduce the post-thawed quality of semen, and plasma membrane of spermatozoa is a key component that must be maintained (Watson, 2000; Aboagla and Terad, 2003).

Reactive oxygen species (ROS) and antioxidants have been shown to play an important role in male fertility. Several evidences suggest reduced antioxidant levels in mammalian sperm and hence they are sensitive to oxidative damage induced by high O_2 concentrations. The plasma membrane of mammalian spermatozoa contains high concentrations of polyunsaturated fatty acids like arachidonic and decosahexaenoic acids, which make it susceptible to ROS induced peroxidative damage with a subsequent loss of sperm functions in buffalo as compared to bovine sperm (Lenzi *et al*., 2002; Nair *et al*., 2006). Consequences of oxidative damage were numerous, ranging from membrane damage, inhibition of respiration, leakage of intrcellular enzymes, axonemal protein damage and mitochondrial membrane damage (Aitken *et al*., 1998). One major focus of damage of these molecules was on mitochondria, leading to loss of sperm motility via alterations in mitochondrial function through ATP depletion (De-lamirande and Gagnon, 1992). Since high mitochondrial membrane potential was required for mitochondrial ATP production and sperm motility. Addition of various antioxidants in the semen extender during liquid storage or cryopreservation may reduce (ROS) production and prevent oxidative stress (Foote *et al*., 2002; Funahashi and Sano, 2005).

Leptin is a 167- amino acid and 16 KDa protein adipokine, a pleiotropic cytokine-like hormone that produced primarily by white adipose tissue, involved in regulation of energy homeostasis, fatty acid oxidation, angiogenesis, puberty and reproduction (Branian and

Hansen 2002; du plessis *et al*., 2010). Leptin is mainly known as a hormonal link between energy stores and energy homeostasis (Cervero *et al*., 2006). Recently, it was reported that sperm had ability to modulate its metabolism by leptin secretion, according to its energy sperm requirements and influences the mechanisms involved in sperm motility development (Aquila *et al*., 2005; Lang Consiglio et al., 2009). In this respect, Jorsaraei *et al*. (2008) reported positive effects of leptin on sperm characteristics. Also, Jorsaraei *et al*. (2010) found a significant correlation between level of leptin in sperm motility. Moreover, Khaki et al. (2013) showed that *in vitro* addition of 10 ng/ml leptin can preserve sperm motility and viability in cooled buffalo semen.

Melatonin (N- actey 1-5 methoxy tryptamine; MW= 232) is secreted by the pineal gland in the brain and plays an important role in regulating the neuroendocrine system. This hormone is one of the major role players in the regulation of the circadian sleep wake cycle (Awad et al., 2006). Some studies have demonstrated that besides its multiple actions on different physiological processes, melatonin as well as its metabolites is indirect antioxidants and powerful direct scavengers of free radicals, and several reports have substantiated the protective effects of melatonin as an antioxidant because of its high efficacy as a hydroxyl radical (OH^-) scavenger (Lee et al., 2002; El-Sokkary *et al*., 2003). Furthermore, melatonin has the ability to detoxify different kinds of ROS and nitrogen species involving singlet oxygen (O_2) (Tan et al., 2002). Addition of melatonin to ram, boar and bull semen has been shown to protect sperm against the harmful effects of ROS and improve sperm motility and membrane integrity during sperm cryopreservation or storage in fresh state (Ashrafi *et al*., 2011; Ashrafi *et al*., 2013).

Therefore, the aim of the current study was to evaluate the possible effects of leptin or melatonin as a protective additive in Tris-extender on frozen semen quality of buffalo bull.

MATERIALS AND METHODS

The present study was conducted at Animal Production Research Station, El-Gemmezah, Gharbiya Governorate, belonging to Animal Production Research Institute, Egypt, in cooperation with Department of Animal Production, Faculty of Agriculture, Mansoura university. The experimental work lasted during the period from April to October, 2014.

Animals:

Five sexually mature Egyptian buffalo bulls (aged 4 to 5 years) with an average live body weight of (450-500 kg) were used in this study. All bulls were free of any diseases with healthy appearance and fed on daily ration composed of 8 kg concentrate fed mixture (CFM), 6 kg berseem hay (BH) and 6 kg rice straw (RS). Feeds were given individually to bulls at 8.00 a.m., while drink clean water available all day time. Animals were housed individually under semi-open sheds. The CFM was composed of 25% undecorticated cotton seed cake, 44% coarse wheat bran, 15% corn, 8.5% extracted rice bran, 3% molasses, 3% limestone and 1.5% sodium chloride.

Semen collection:

Semen was collected, twice a week from five bulls for 4 weeks (40 ejaculates for each bulls), using artificial vagina (IMV, France) maintained at appropriate temperature degrees. Semen was collected before feeding from 7.00 a.m. to 8.00 a.m. A bull was used as a teaser on day of semen collection. The collected ejaculates were put in water bath at 37 ^{0}C for evaluation and freezing processes only for ejaculates with mass motility of more than 70% on day of semen collection. All ejaculates were pooled and divided into 7 replicates for different treatments (3 levels of melatonin or leptin and control).

Experimental semen extenders:

Semen was extended in Tris- egg yolk extender (Tris, 0.325 g; Citric acid, 1.675 g; Glucose, 0.75 g; Streptomycin, 0.005 g and Lincomycin, 0.25 g in 100 ml distilled water, then 10% egg yolk and 7% glycerol were added to 83 ml Tris extender), then Tris-extender was divided into 7 portions. The 1st treatment of Tris extended was unsupplemented (T1) and served as control, while in T2, T3 and T4, leptin (Sigma Aldrich co., St Louis, Mo, USA) was added to Tris-extender at levels of 10, 20 50 ng/ml and in T5, T6 and T7, melatonin (Sigma Aldrich co., St Louis , Mo, USA) was added at levels of 10^{-3}M, 10^{-6}M and 10^{-9}M, respectively. The dilution rate was at a rate of 1:10.

Semen processing:

The diluted semen of each treatment was aspirated into medium-sized (0.25 ml) French straws, closed with polyvinyl alcohol power and equilibrated at 5^{0}C for 4 h. After equilibration, the straws were frozen in liquid nitrogen vapour, 5 cm above liquid nitrogen surface, for 10 min and then the straws were plunged into liquid nitrogen (-196^{0}C) for storage. After storage for 4 weeks, frozen straws were thawed at 37^{0}C for 30 s in a water bath.

Semen evaluation:

Semen extended with each treatment level was evaluated in post-dilution, post-equilibrated and post-thawed semen. Various sperm parameters including percentage of individual motility, dead sperm and sperm abnormality were determined, using a hot microscope stage adjusted at 37 ^{o}C. The percentage of individual motility was measured using research microscope with warmed stage (37°C) under the high power magnification (x400) according to Amman and Hammerstedt (1980). Dead sperm percentage was determined using eosin and nigrosin mixture stain according to Hackett and Macpherson (1965). The percentage of sperm total abnormalities was determined during the examination of live/dead sperm percentage at a high power magnification (400x), according to the classification adopted by Blom (1983).

Enzyme activity in seminal plasma:

After semen thawing, the seminal plasma was separated by centrifugation (4000 rpm, for 15 min) and stored at -20 ^{o}C until analysis. Activity of asprtate (AST) and alanine (ALT) transaminases as well as lactic dehydrogenase (LDH) in seminal plasma was measured commercial kits Salucea Netherlands (Young, 1990) and spectrophotometer (JENWAY-6405UV/Vis).

Fertility trail:

About 48-72 h before artificial insemination, buffalo cows were i.m. injected with 3 ml Estrumate (PGF2α-Essex Animal Helth Friesoythe, Germany) to induce estrus. Total of 12 buffalo cows in heat were inseminated by semen extended with control semen (T1) and the best level of leptin and melatonineach, based on the obtained results. Animals were immediately inseminated post-thawing (at a rate of 37 ºC for 30 seconds) using filled plastic AI gun close to the cervix. Pregnancy rate was measured by rectal palpation on day 50 after (AI). All inseminations were conducted by the same inseminator.

Statistical analysis:

Data were statistically analyzed by the methods of analysis of variance according model procedures of SPSS (2013). Duncan multiple rang test was used to test the differences among means (Duncan, 1955). The percentage values 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

Sperm parameters in post-diluted semen:

Percentages of progressive sperm motility and sperm abnormality significantly (P<0.05) improved by adding 20 ng/ml of leptin or 10^{-3} M of melatonin in Tris-extender. However, percentage of dead sperm significantly (P<0.05) improved by all additives as compared to unsupplemented extender. Generally, addition of 20 ng/ml of leptin showed significantly (P<0.05) the best results concerning sperm characteristics studied in post-diluted as compared to other additives and unsupplemented extenders (Table 1)

Table (1): Effect of leptin and melatonin supplementation to Tris-extender on sperm parameters in post-diluted buffalo semen.

Sperm characteristics (%) in post-diluted semen	Control	Leptin (ng/ml)			Melatonin (M)			Overall
		10	20	50	10^{-3}	10^{-6}	10^{-9}	
Sperm motility	68.12^{c}	70.00^{c}	76.75^{a}	69.87^{c}	74.00^{b}	69.75^{c}	69.12^{c}	71.08
	±0.61	±0.50	±0.94	±1.10	±0.65	±0.55	±0.47	±0.46
Dead sperm	30.00^{a}	22.25^{d}	17.00^{f}	25.00^{c}	19.50^{e}	25.12^{c}	27.12^{b}	23.71
	±1.29	±1.03	±0.84	±1.22	±0.98	±1.28	±1.30	±0.69
Sperm abnormality	20.00^{a}	17.37^{a}	12.00^{b}	18.12^{a}	13.75^{b}	18.37^{a}	19.25^{a}	16.98
	±0.98	±0.67	±0.65	±1.00	±0.59	±0.90	±1.03	±0.47

Means denoted within the same row with different superscripts are significantly different at P<0.05.

Sperm characteristics in post-equilibrated semen:

Sperm characteristics including percentages of progressive sperm motility, dead sperm and sperm abnormality showed significantly (P<0.05) the best results in post-equilibrated semen diluted with 20 ng/ml of leptin, followed by that was diluted with 10^{-3} M of melatonin in Tris-extender in comparison with other additives and unsupplemented extenders (Table 2).

Table (2): Effect of leptin and melatonin supplementation to Tris-extender on sperm parameters in post-equilibrated buffalo semen.

Sperm characteristics (%) in post-equilibrated semen	Control	Leptin (ng/ml)			Melatonin (M/ml)			Overall
		10	20	50	10^{-3}	10^{-6}	10^{-9}	
Sperm motility	63.00^{d}	65.12^{cd}	75.62^{a}	64.25^{cd}	71.25^{b}	66.00^{c}	63.75^{cd}	67.00
	±0.84	±0.71	±1.06	±0.81	±0.52	±1.13	±0.95	±0.66
Dead sperm	34.37^{a}	26.75^{e}	19.12^{f}	30.12^{bc}	23.50^{b}	29.12^{cd}	32.50^{b}	27.92
	±1.37	±1.31	±0.83	±0.54	±0.77	±0.63	±0.77	±0.74
Sperm abnormality	25.00^{a}	21.25^{b}	14.12^{c}	23.12^{ab}	16.00^{c}	25.37^{a}	24.62^{a}	21.35
	±1.29	±0.79	±0.71	±1.12	±0.63	±0.53	±0.37	±0.64

Means denoted within the same row with different superscripts are significantly different at P<0.05.

Sperm characteristics in post-thawed semen:

As found in post-diluted and post-equilibrated semen, sperm characteristics involving percentages of progressive sperm motility, dead sperm and sperm abnormality also significantly (P<0.05) improved in post-thawed semen diluted with 20 ng/ml of leptin or 10^{-3} M of melatonin in Tris-extender, being better with leptin more than with melatonin (Table 3).

Table (3): Effect of leptin and melatonin supplementation to Tris-extender on sperm parameters in post-thawed buffalo semen.

Sperm characteristics (%) in post-thawed semen	Control	Leptin (ng/ml)			Melatonin (M/ml)			Overall
		10	20	50	10^{-3}	10^{-6}	10^{-9}	
Sperm motility	41.25^{d}	46.37^{c}	59.25^{a}	44.50^{cd}	54.62^{b}	45.00^{cd}	43.25^{cd}	47.75
	±1.95	±1.49	±0.75	±1.73	±1.86	±1.41	±1.60	±1.00
Dead sperm	56.25^{e}	50.12^{c}	38.37^{a}	52.73^{d}	45.00^{b}	49.62^{c}	51.12^{cd}	48.98
	±1.72	±1.02	±1.37	±1.14	±1.41	±1.16	±0.95	±0.85
Sperm abnormality	41.25^{e}	32.37^{c}	20.00^{a}	36.62^{d}	23.12^{b}	36.87^{d}	37.37^{d}	32.51
	±1.95	±1.11	±0.92	±1.73	±1.00	±1.83	±1.86	±1.13

Means denoted within the same row with different superscripts are significantly different at P<0.05.

Enzyme activity in seminal plasma of post-thawed semen:

Enzyme activity of AST, ALT and LDH significantly (P<0.05) reduced in seminal plasma of post-thawed semen diluted with all additives as compared to unsupplemented semen, being the lowest significantly (P<0.05) in seminal plasma of semen diluted with 20 ng/ml of leptin (Table 4).

Table (4): Effect of leptin and melatonin supplementation to Tris-extender on enzyme activity in seminal plasma of post-thawed buffalo semen.

Enzyme activity in seminal plasma (U/L)	Control	Leptin (ng/ml)			Melatonin (M/ml)			Overall
		10	20	50	10^{-3}	10^{-6}	10^{-9}	
AST	98.75^{a} ±1.49	64.50^{d} ±1.70	58.50^{e} ±0.64	64.75^{cd} ±1.03	58.75^{e} ±2.05	68.50^{bc} ±0.64	69.25^{b} ±0.47	69.00 ±2.49
ALT	59.25^{a} ±1.31	45.75^{bc} ±0.47	37.75^{d} ±1.11	46.00^{b} ±0.40	43.25^{c} ±1.11	46.50^{b} ±0.28	46.50^{b} ±0.64	46.42 ±1.18
LDH	392.50^{a} ±3.12	350.50^{b} ±2.10	316.25^{d} ±4.03	351.75^{b} ±2.56	330.50^{c} ±3.37	355.50^{b} ±1.55	356.75^{b} ±1.37	350.53 ±4.34

Means denoted within the same row with different superscripts are significantly different at P<0.05.

Fertility trail:

In association with improving different sperm characteristics studied by adding leptin or melatonin at levels of 20 ng/ml and 10^{-3} M, respectively, results of fertility trail indicated that buffalo cows inseminated with semen extended with Tris-extender supplemented with 20 ng/ml of leptin showed significantly (P<0.05) higher conception rate (CR, 91.6%) than those inseminated by semen supplemented with 10^{-3} M of melatonin (75.0%) or unsupplemented semen (66.6%). Also, addition of 10^{-3} M of melatonin to extended semen improved CR as compared to unsupplemented semen, but the difference was not significant (75.0 vs. 66.6%).

Table (5):Conception rate of buffalo cows artificially inseminated with frozen semen extended with leptin (20 ng/ml), melatonin (10^{-3} M) and unsupplemented one.

Item	Frozen semen		
	Control (unsupplemented)	Leptin (20 ng/ml)	Melatonin (10^{-3}M)
Number of inseminated animals	12	12	12
Number of conceived animals	8	11	9
Conception rate (%)*	66.6^{b}	91.6^{a}	75.0^{b}

Means denoted within the same row with different superscripts are significantly different at P<0.05.

*** Conception rate (%) of buffalo cows within 60 days postpartum**

DISCUSSION

Using artificial insemination is one of the most important tools in buffalo farms to accelerate genetic improvement (AI). AI plays a potential role in terms of health and economic production and the most important need is high quality semen from a proven fertile bulls. Leptin in the normal values has positive effects on male reproductive activity, but increasing leptin secretion from adipose tissue is known to have a deleterious effect on spermatogenesis and androgens secretion by Leydig cells (Tena-Sempere and Barreiro, 2002). Several authors indicated leptin presence in the seminal plasma of human (Glander et al., 2002 and Lackey et al., 2002), which may lead to the original suggestion that leptin present in the seminal plasma is mainly secreted from the accessory sex glands. It was indicated that the localization of a 145-kDa leptin receptor isoform to the tail region of ejaculated spermatozoa and spermatozoa with deteriorated membranes contained significantly less leptin receptor (Jope et al., 2003). The expression of leptin and leptin receptors on human spermatozoa by immunofluorescent staining (Li et al., 2008), and leptin is expressed in and secreted from human ejaculated spermatozoa (Aquila et al., 2005). These findings may suggest that leptin present in the seminal plasma may exert a local function on spermatozoa and may regulate sperm function. It was reported that the increased serum leptin might directly affect testicular function to reverse spermatogenic dysfunction (.14,22,31,40Contrary, There was a negative signification between seminal leptin and sperm motility (Jorsaraei et al., 2010). Also, Glander *et al*. (2002) reported that seminal plasma leptin levels were significantly lower in patients with normal spermiogram parameters, compared with pathological semen samples suggesting that higher leptin concentration had negative effects on sperm function. Furthermore, many authors reported a negative correlation of leptin concentration in seminal plasma with the percentage of motile spermatozoa and the straight line velocity of human spermatozoa (Glander et al., 2002; von Sobbe et al., 2003; Jorsaraei *et al*., 2010*)*. However, some investigators found no correlation between seminal plasma leptin and physical characteristics of semen samples In this way, Camina et al. (2002) found leptin concentration in seminal plasma had no correlation with semen characteristics such as sperm cell concentration, sperm motility, vitality or morphology. Also, However, Zorn *et al*. (2007) reported no correlation between leptin levels and sperm motility or morphology. They proposed that the increased serum leptin might directly affect testicular function to reverse spermatogenic dysfunction.

Some studies were conducted about the effect of leptin on semen production in men and some laboratory animals, reporting controversial results. Aim of the present study was emphazied that adding leptin at different levels into the buffalo semen extender to evaluate *in vitro* effect of leptin on motility, livability, and abnormality of buffalo spermatozoa in diluted, equilibrated and frozen/thawed semen. The obtained results from the present study showed that exogenous

leptin at a level of 20 ng/ml to the extender of frozen buffalo semen was unlikely to act on sperm function directly. In accordance with the present results, Khaki et al. (2013) suggested the probable effects of leptin addition (0, 10, 20, 50, 100, and 200 ng mL-1) to Tris-extender of water buffalo frozen semen on sperm quality (motility and motility parameters, viability, sperm membrane integrity, and DNA damage). They showed that addition of 10 ng mL-1 leptin into semen extender significantly preserved sperm motility, all of the motility parameters, and viability in equilibrated semen compared to that of control and other levels of leptin. Adding leptin to semen extender did not have any significant influence on sperm DNA damage and sperm membrane integrity. They suggested that *in vitro* addition of 10 ng/mL leptin could preserve sperm motility and viability in cooled semen of buffaloes. Also, Lampiao and du Plessis (2008) found that *in vitro* leptin significantly increased total motility, progressive motility and acrosome reaction as well as nitric oxide production in human spermatozoa. They concluded that the hormone could play a role in enhancing the fertilization capacity of human spermatozoa via increasing motility and acrosome reaction. Moreover, Jorsaraei *et al*. (2008) reported that administration of 30 ng/mL leptin had a positive but not significant effect on human sperm motility after 4 h incubation. Moreover, Lampiao and du Plessis, 2008) reported that leptin played a role in enhancing human sperm motility parameters, as evidenced by increased total and progressive motility as well as the sperm hyperactivation characteristics. On the other hand, Li *et al*. (2009) found no significant difference in sperm motility parameters determined and percentages of capacitated and acrosome-reacted spermatozoa.

Leptin secretion was reported to be significantly increased in capacitated sperm than in non-capacitated sperm, suggesting the involvement of this hormone in capacitation. It was not known whether prior action of leptin on the spermatozoa could have a priming effect on the subsequent oocyte activation and early embryo development (Li et al., 2009). The physiological role of leptin in improving sperm characteristics may be due to that leptin treatment stimulated cholesterol efflux and acrosin activity (protein tyrosine phosphorylation) in spermatozoa. In pig, Aquila et al. (2008) reported that when washed pooled human spermatozoa from normal samples were treated with leptin and incubated under uncapacitating condition. They hypothesized an action of leptin in modulating sperm energetic substrate availability during capacitation. Also, leptin played roles in spermatogenesis or sperm capacitation, and facilitate ovarian cycle (Garcia-Mayor et al., 1997; Bado et al., 1998). These findings may indicate the beneficial effect of leptin of conception rate of buffalo cows in our study

In addition, the obtained results from our study revealed impact of exogenous addition of melatonin at a level of 10^{-3} M in frozen buffalo semen. In this respect, Gavella and Lipovac (2000) studied the ability of melatonin to counteract lipid peroxidation in human sperm. They reported a significant inhibitory effect of melatonin on further propagation of iron-induced lipid peroxidation in the sperm membrane. Melatonin significantly inhibited rate of lipid peroxidation in vitro. Thus, one may suppose that melatonin could protect against further diffusion of peroxidative damage in the sperm membrane if ferrous ions are present in situation *in vivo* (Kwenang et al., 1987). If the concentration of melatonin in human semen was found to be lower than nanomolar range (Bornman et al., 1989), there was high possibility that exogenous melatonin has some potential in correcting the influence of oxidative stress on human sperm. It was of interest to note that improving sperm characteristics by leptin (20 ng/ml) or melatonin (10^{-3} M), during freezing process was associated with marked reduction in release of enzymes like AST, ALT and LDH and reflected higher conception rate, significantly with leptin and insignificantly with melatonin in comparison with unsupplemented semen.

CONCLUSION

In conclusion, the obtained results revealed that adding 20 ng/mL leptin and 10^{-3} M melatonin to Tris-based extender had beneficial effects on motility, livability and normality of buffalo spermatozoa in diluted, equilibrated and thawed semen as compared to unsupplemented semen. These improvements was associated with increasing conception rate of buffalo cows which can be helpful for artificial insemination. Leptin may affect capacitation and hyperactivation of sperm in equilibrated and thawed semen.

REFERENCES

Aboagla, E.M.E. and Terad, T. (2003). Trehalose enhanced fluidity of the goat sperm membrane and its protection during freezing. Biol. Reprod., 69: 1245-1250.

Aitken, R.J.; Gordon, E.; Harkiss, D.; Twigg, J.P.; Milne, P.; Jennings, Z. and Irvine, D.S. (1998). Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. Biol. Reprod., 59: 1037–1046.

Amman, R.P. and Hammerstedt, R.H. (1980). Validation of a system for computerized measurements of spermatozoa velocity and perce ntage of motile sperm. Biol. Reprod., 23: 647-656.

Andrabi, S.M.H. (2009). Factors Affecting the Quality of Cryopreserved Buffalo (Bubalus bubalis) Bull Spermatozoa. Reprod. Dom. Anim., 44: 552-569.

Aquila, S.; Gentile, M.; Middea, M.; Catalano, S.; Morelli, C. and Pezzi, V. (2005). Leptin secretion by human ejaculated spermatozoa. J. Clin. Endocrinol. Metab., 90: 4753-4761.

Aquila, S.; Rago, V. and Guido C. (2008). Leptin and leptin receptor in pig spermatozoa: Evidence of their involvement in sperm capacitation and survival. Reprod ., 136(1): 23-32.

Ashrafi, I. ; Kohram, H. ; Naijian, H.; Bahreini, M. and Poorhamdollah, M. (2011). Protective effect of melatonin on sperm motility parameters on liquid storage of ram semen at 5°C. Afri. J. Biotech., 10(34): 6670–6674.

Ashrafi, I.; Kohram, H. and Ardabili, F.F. (2013). Antioxidative effects of melatonin on kinetics, microscopic and oxidative parameters of cryopreserved bull spermatozoa. Anim. Reprod. Sci., 139: 25-30.

Awad, H.; Halawa, F.; Mostafa, T. and Atta, H. (2006). Melatonin hormone profile in infertile males. Int. J. Androl., 29: 409-413.

Bado, A.; Levasseur, S.; Attoub, S.; Kermorgant, S.; Laigneau, J.P. and Bortoluzzi, M.N. (1998). The stomach is a source of leptin. Nature ., 20: 790-793.

Blom, E. (1983). The spermiogram of the bull. Nordisk Veterinear Medicin., 35 : 105.

Bornman, M.S.; Oosthuizen, J.M.C.; Barnard, H.C.; Sculenberg, G.W.; Boomker, D. and Reif, S. (1989). Melatonin and sperm motility. Andrologia., 21: 483–485.

Branian, J.D. and Hansen, k.A. (2002). Leptin and ovarian folliculogenesis: Implicationsfor ovulation induction and ART outcomes. Semin. Reprod. Med., 20: 103-112.

Bucak, M. N.; Sarıözkan, S.; Tuncer, P.B.; Ulutaşand, P.A. and Akçadağ, H.I. (2009). Effect of antioxidants on microscopic semen parameters, lipid peroxidation and antioxidant activities in Angora goat semen following cryopreservation. Small Rum. Res., 81: 90-95.

Camina, J.P.; Lage, M.; Menendez, C.; Grana, M.; Garcia-Devesa, J. and Dieguez, C. (2002). Evidence of free leptin in human seminal plasma. Endocrine., 17: 169-174.

Cervero, A.; Dominguez, F.; Horcajadas, J.A.; Quinonero, A.; Pellicer, A. and Simon.C.(2006). The role of the leptin in reproduction. Current Opinion in Obstetrics and Gynecology.,18:297-303.

De Lamirande, E. and Gagnon, C. (1992). Reactive oxygen species and human spermatozoa I. Effects on the motility of intact sperma-tozoa and on sperm axonemes. J. Androl., 13: 368–378.

Du Plessis, S.S.; Cabler, S. and McAlister, D.A. (2010). The effect of obesity on sperm disorders and male infertility. Nat. Rev. Urol., 7: 153-161.

Duncan, D.B. (1955). Multiple Range and Multiple F. Test Biometrics., 11: 1-42.

El-Sokkary, G.H.; Kamel, E.S. and Reiter, R.J. (2003). Prophylactic effect of mela-tonin in reducing lead-induced neurotoxicity in the rat. Cell. Mol. Biol. Lett., 8: 461–470.

FAO, (2004). Food and Agricultural Organization, 2004, FAO-STAT, Global Livestock Production and Health Atlas. Animal Production and Health Division, Rome, Italy Available at: (http://www.fao.org/ag/aga/glipha/in-dex.jsp).

Foote, R.H.; Brokett, C.C.; Kaproth, M.T. (2002). Motility and fertility o bull sperm in whole milk extender containing antioxidants. Anim. Reprod. Sci., 71: 13–23.

Funahashi, H. and Sano, T. (2005). Select antioxidants improve the function of extender boar semen at 100C. Theriogenology., 63: 1605-1616.

Garcia-Mayor, R.V.; Andrade, M.A.; Rios, M.; Lage, M.; Dieguez, C. and Casanueva, F.F. (1997). Serum leptin levels in normal children: relationship to age, gender, body mass index, pituitary-gonadal hormones and pubertal stage. J. Clin. Endocrinol Metab., 82: 4144-4148.

Gavella, M. and Lipovac, V. (2000). Antioxidative effect of melatonin on human spermatozoa. Arch. Androl., 44: 23–27.

Glander, H.J.; Lammert, A.; Paasch, U.; Glasow, A. and Kratzsch, J. (2002). Lepin exits in tubuli seminiferi and in seminal plasma. Andrologia ., 34: 227-233.

Hackett, A.J. and Macpherson, J.W. (1965). Some staining procedures for spermatozoa. A review. Can. Vet. J., 5 : 55.

Jope, T.; Lammert, A. and Kratzsch, J. (2003). Leptin and leptin receptor in human seminal plasma and in human spermatozoa. Int. J. Androl., 26: 335-341.

Jorsaraei, S.G.A.; Shibahara, H.; Ayustawait; Hirano, Y.; Shiraishi, Y.; Khalatbari, A.; Pasha, Y.Y. and Suzuki, M. (2008). The in-vitro effects of nicotine, cotinine and leptin on sperm parameters analyzed by CASA system. Iranian J. Reprod. Med., 6(3): 157-165.

Jorsaraei, S.G.A.; Shibahara, H.; Ayustawait; Hirano, Y.; Suzuki, T.; Marzony, E.T.; Zainalzadeh, M. and Suzuki,M. (2010). The Leptin concentrations in seminal plasma of men and its relationship to semen parameters. Iranian J. Reprod. Med., 8(3): 95-100.

Khaki, A.; Batavani, R.A. and Najafi,G. (2013). The in vitro effect of leptin on semen quality of water buffalo (Bubalus bubalis) bulls. Veterinary Research Forum., 4(1): 7-12.

Kumar, R.; Mohanarao, J.; Arvind. and Atreja, S.K. (2011). Freeze–thaw induced genotoxicity in buffalo (Bubalus bubalis) spermatozoa in relation to total antioxidant status. Mol. Biol. Rep., 38: 1499-1506.

Kwenang, A.; Kross, M.J.; Koster, J.F. and van Eyk, H.G. (1987). Iron, ferritin and cooper in seminal plasma. Hum. Reprod., 2: 387–388.

Lackey, B.R.; Gray, S.L. and Henricks, D.M. (2002). Measurement of leptin and insulin-like growth factor-I in seminal plasma from dif-ferent species. Physiological Research., 51: 309–311.

Lampiao,F. and Du plessis, S.S. (2008). Insulin and leptin enhance human sperm motility, acrosome reaction and nitric oxide production. Asian. J. Androl., 10 (5): 799–807.

Lange Consiglio, A.; Dell'Aquila, M.E.; Fiandanese, N.; Ambruosi, B.; Cho, Y.S. and Bosi, G. (2009). Effects of leptin on in vitro maturation, fertilization and embryonic cleavage after ICSI and early developmental expression of leptin (Ob) and leptin receptor (ObR) proteins in the horse. Reprod. Biol. Endocrinol., 7:113.

Leboeuf, B.; Restall, B. and Salamon, S. (2000). Production and storage of goat semen for artificial insemination. Anim. Reprod. Sci., 62: 113-141.

Lee, Y.M.; Chen, H.R.; Hsiao, G.; Sheu, J.R.; Wang, J.J. and Yen, M.H. (2002). Protec-tive effects of melatonin on myocardial ischemia/reperfusion injury in vivo. J. Pineal Res., 33: 72–80.

Lenzi, A.; Gandini, L.; Lombardo, F.; Picardo, M.; Maresca, V. and Panfili, E. (2002). Polyunsaturated fatty acids of germ cell membranes, glutathione and glutathione-dependent enzyme-PHGPx: from basic to clinic. Contraception., 65: 301-304.

Li, HW.; Chiu, P.C.; Cheung, M.P.L. and Yeung,W.S.B. (2008). Effect of leptin on motility, capacitation and acrosome reaction of human spermatozoa. Int. J. Androl., 32: 687-694.

Nair, J.S.; Brar, A.S.; Ahuja, C.C.; Sangha, S.P.S. and Chaudhary, K.C. (2006). A comparative study on lipid peroxidation, activities of antioxi-dant enzymes and viability of cattle and buffalo bull spermatozoa during storage at refrigeration temperature. Anim. Reprod. Sci., 96: 21-29.

S.P.S.S. (2013). SPSS user Guide: Statistics SPSS.

Tan, D.X..; Reiter, R.J.; Manchester, L.C.; Yan, M.T.; El-Sawi, M.; Sainz, R.M.; Mayo, J.C.; Kohen, R.; Allegra, M. and Hardeland, R. (2002). Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger. Curr. Top. Med. Chem., 2: 181–197.

Tena-Sempere, M. and Barreiro, M.L. (2002). Leptin in male reproduction: the testis paradigm. Mol. Cell. Endocrinol., 188: 9-13.

Von Sobbe, H.U.; Koebnick, C. and Jenne, L. (2003). Leptin concentrations in semen are correlated with serum

leptin and elevated in hypergonadotrophic hypo-gonadism. Andrologia ., 35: 233-237.

Watson, P.F. (2000). The causes of reduced fertility with preserved semen. Anim. Reprod. Sci., 60: 481-492.

Young, D.S. (1990). Effects of drugs on clinical laboratory tests. Third edition., 3: 6-12.

Zorn, B.; Osredkar, J. and Meden-Vrtovec, H. (2007). Leptin levels in infertile male patients are correlated with inhibin B, testosterone and SHBG but not with sperm characteristics. Int. J. Androl., 30: 439-444.

تأثير اللبتين والميلاتونين كإضافات حماية لمخفف الترس علي جودة السائل المنوي المجمد لطلائق الجاموس

عبد الخالق السيد عبد الخالق[١]، حمدى عبد الله النجار[٢]، أميمة مصطفى ابراهيم[٢]

١ -قسم الانتاج الحيوانى،كلية الزراعة ،جامعة المنصورة،مصر.

٢ - معهد بحوث الانتاج الحيوانى ،مركز البحوث الزراعية ،وزارة الزراعة ، مصر.

أجريت هذه الدراسة في محطة بحوث الانتاج الحيواني بالجميزة ، التابعة لمعهد بحوث الإنتاج الحيواني ،مصر و بالتعاون مع قسم الإنتاج الحيواني ، كلية الزراعة ، جامعة المنصورة . والهدف من الدراسة الحالية هو تقييم التأثيرات المحتملة لكلا من اللبتين والميلاتونين كإضافات حماية لمخفف الترس على جودة السائل المنوى المجمد لطلائق الجاموس. إستخدم في هذه الدراسة 5 طلائق جاموس ناضجة جنسيا يبلغ عمرها (4 - 5) سنوات ، وكان متوسط الوزن أثناء التجارب العملية يتراوح ما بين (450 –500) كجم وتمت تغذيتهم على عليقة موحدة ، تم جمع السائل المنوي من الطلائق بواسطة المهبل الاصطناعي مرتين أسبوعينا و لمدة أربعة أسابيع (40 قذفة لكل الطلائق) و كانت النسبة المئوية للحركة الجماعية للحيوانات المنوية من القذفات المجمعة 70% أو أكثر ثم خلطت وقسمت الي سبعة مقرارت للمعاملات المختلفة وهى: المعاملة الاولى (مخفف الترس وصفار البيض بدون اضافة وهى كنترول) ، المعاملة الثانية والثالثة والرابعة (اضافة اللبتين بمستويات 10 , 20 و 50 نانو جرام / مل)، المعاملة الخامسة والسادسة والسابعة (اضافة الميلاتونين بمستويات 10^{-3}, 10^{-6}, 10^{-9} مول) تقريبا. وتم وضع السائل المنوى المخفف فى فترة موازنة على 5 درجة مئوية لمدة 4 ساعات ثم تجميده فى النتروجين السائل وبعد الحفظ لمدة 4 أسابيع أسيلت قصيبات السائل المنوي المجمده لكل المعاملات عند37 درجة مئوية لمدة 30 ثانية،وتم عمل تقييم للسائل المنوى بعد التخفيف وبعد فترة الموازنة وبعد الاسالة.قدرت خصائص السائل المنوي المختلفة كنسبة مئوية (%) بعد التخفيف وبعد فترة الموازنة وبعد الاسالة وهي (الحركة الفردية ، الحيوانات المنوية الميتة ، الشواذ للحيوانات المنوية). تم تقدير نشاط الانزيمات فى بلازما السائل المنوى وتشمل (AST, ALT, LDH) بعد عملية التجميد والاسالة ، وتم تقدير معدل الحمل بعد أن لقحت 12 أنثى من الجاموس صناعيا باستخدام مخففات (المعاملة الاولى- الثالثة – الخامسة) وتم جس الاناث بعد حوالى ٥٠ يوم من التلقيح الصناعى. وأشارت النتائج إلي تحسن نسبة (الحركة الفردية و الشواذ)عند مستوي معنوية (٠.٠٥ %) لكل من اللبتن ٢٠ نانوجرام / مل و الميلاتونين M 10^{-3} وكذلك تحسين في نسبة الحيوانات المنوية الميتة في كل المعاملات مقارنة بالكنترول ، اللبتن ٢٠ نانوجرام / مل أظهر تحسن في نتائج خصائص السائل المنوي المقيمة بعد التخفيف مقارنة بالمعاملات الاخري والكنترول ، وكذلك أشارت النتائج إلي تحسن في نسبة (الحركة الفردية ، الحي والميت و الشواذ) مع اللبتن ٢٠ نانوجرام / مل يليه الميلاتونين 10^{-3} Mبعد فترة الموازنة و بعد ألاسالة مقارنة بالمعاملات الاخري والكنترول. أشارت النتائج نقص في النشاط الانزيمي AST,ALT,LDH بعد الاسالة في كل المعاملات مقارنة بالكنترول وكانت منخفضة مع اللبتن ٢٠ نانوجرام / مل عند مستوي معنوية (٠.٠٥ %). أشارت النتائج إلي أن أعلي نسبة مئوية لمعدل الحمل كان مع اللبتن ٢٠ نانوجرام / مل يليه الميلاتونين M 10^{-3} مقارنة بالكنترول (٩١.٦ ، ٧٥.٠ و ٦٦.٦ % علي التوالي). ونستخلص من هذه الدراسة أن إضافة أى من اللبتين والميلاتونين إلى مخفف الترس كإضافات حماية قد أدى إلى زيادة جودة السائل المنوى المجمد لطلائق الجاموس.