

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

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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°C for 4 h and frozen in liquid nitrogen. After storage for 4 weeks, frozen straws were thawed at 37°C 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 O₂ 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 intracellular 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-acetyl 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 (O₂) (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 °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 10⁻³M, 10⁻⁶M and 10⁻⁹M, 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 powder and equilibrated at 5°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°C) for storage. After storage for 4 weeks, frozen straws were thawed at 37°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 °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 °C until analysis. Activity of aspartate (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 (PGF2 α -Essex Animal Health 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 melatonin each, based on the obtained results. Animals were immediately inseminated post-thawing (at a rate of 37 °C 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 ±0.61	70.00 ^c ±0.50	76.75 ^a ±0.94	69.87 ^c ±1.10	74.00 ^b ±0.65	69.75 ^c ±0.55	69.12 ^c ±0.47	71.08 ±0.46
Dead sperm	30.00 ^a ±1.29	22.25 ^d ±1.03	17.00 ^f ±0.84	25.00 ^c ±1.22	19.50 ^e ±0.98	25.12 ^c ±1.28	27.12 ^b ±1.30	23.71 ±0.69
Sperm abnormality	20.00 ^a ±0.98	17.37 ^a ±0.67	12.00 ^b ±0.65	18.12 ^a ±1.00	13.75 ^b ±0.59	18.37 ^a ±0.90	19.25 ^a ±1.03	16.98 ±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 ±0.84	65.12 ^{cd} ±0.71	75.62 ^a ±1.06	64.25 ^{cd} ±0.81	71.25 ^b ±0.52	66.00 ^c ±1.13	63.75 ^{cd} ±0.95	67.00 ±0.66
Dead sperm	34.37 ^a ±1.37	26.75 ^e ±1.31	19.12 ^f ±0.83	30.12 ^{bc} ±0.54	23.50 ^b ±0.77	29.12 ^{cd} ±0.63	32.50 ^b ±0.77	27.92 ±0.74
Sperm abnormality	25.00 ^a ±1.29	21.25 ^b ±0.79	14.12 ^c ±0.71	23.12 ^{ab} ±1.12	16.00 ^c ±0.63	25.37 ^a ±0.53	24.62 ^a ±0.37	21.35 ±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 ±1.95	46.37 ^c ±1.49	59.25 ^a ±0.75	44.50 ^{cd} ±1.73	54.62 ^b ±1.86	45.00 ^{cd} ±1.41	43.25 ^{cd} ±1.60	47.75 ±1.00
Dead sperm	56.25 ^e ±1.72	50.12 ^c ±1.02	38.37 ^a ±1.37	52.73 ^d ±1.14	45.00 ^b ±1.41	49.62 ^c ±1.16	51.12 ^{cd} ±0.95	48.98 ±0.85
Sperm abnormality	41.25 ^e ±1.95	32.37 ^c ±1.11	20.00 ^a ±0.92	36.62 ^d ±1.73	23.12 ^b ±1.00	36.87 ^d ±1.83	37.37 ^d ±1.86	32.51 ±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 ^c ±0.64	64.75 ^{cd} ±1.03	58.75 ^c ±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 (.14,22,31,40). Contrary, 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 spermogram 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 emphasized 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.

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

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أجريت هذه الدراسة في محطة بحوث الانتاج الحيواني بالجميزة، التابعة لمعهد بحوث الانتاج الحيواني، مصر وبالتعاون مع قسم الانتاج الحيواني، كلية الزراعة، جامعة المنصورة. والهدف من الدراسة الحالية هو تقييم التأثيرات المحتملة لكلا من اللبتين والميلاتونين كإضافات حماية لمخفف الترس على جودة السائل المنوي المجمد لطلائق الجاموس. استخدم 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 أسبوعيا ولمدة أربعة أسابيع (40 قذفة لكل الطلائق) وكانت النسبة المئوية للحركة الجماعية للحيوانات المنوية من القذفات المجمعة 70% أو أكثر ثم خلطت وقسمت الي سبعة مقرارات للمعاملات المختلفة وهي: المعاملة الاولى (مخفف الترس وصفار البيض بدون اضافة وهي كنترول)، المعاملة الثانية والثالثة والرابعة (إضافة اللبتين بمستويات 10، 20 و 50 نانو جرام / مل)، المعاملة الخامسة والسادسة والسابعة (إضافة الميلاتونين بمستويات 10^{-3} ، 10^{-6} ، 10^{-9} مول) تقريبا. وتم وضع السائل المنوي المخفف في فترة موازنة على 5 درجة مئوية لمدة 4 ساعات ثم تجميده في النتروجين السائل وبعد الحفظ لمدة 4 أسابيع أسيلت قصبيا السائل المنوي المجمد لكل المعاملات عند 37 درجة مئوية لمدة 30 ثانية، وتم عمل تقييم للسائل المنوي 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 في بلازما السائل المنوي وتشمل (AST, ALT, LDH) بعد عملية التجميد والاسالة، وتم تقدير معدل الحمل بعد أن لحدت 12 أنثى من الجاموس صناعيا باستخدام مخففات (المعاملة الاولى- الثالثة - الخامسة) وتم جس الاناث بعد حوالي 50 يوم من التلقيح الصناعي. وأشارت النتائج إلي تحسن نسبة (الحركة الفردية و الشواذ) عند مستوي معنوية (0.05%) لكل من اللبتين 20 نانوجرام / مل و الميلاتونين 10^{-3} M وكذلك تحسین في نسبة الحيوانات المنوية الميتة في كل المعاملات مقارنة بالكنترول، اللبتين 20 نانوجرام / مل أظهر تحسن في نتائج خصائص السائل المنوي المقيمة بعد التخفيف مقارنة بالمعاملات الاخرى والكنترول، وكذلك أشارت النتائج إلي تحسن في نسبة (الحركة الفردية، الحي والميت و الشواذ) مع اللبتين 20 نانوجرام / مل يليه الميلاتونين 10^{-3} M بعد فترة الموازنة و بعد الاسالة مقارنة بالمعاملات الاخرى والكنترول. أشارت النتائج نقص في النشاط الانزيمي AST,ALT,LDH بعد الاسالة في كل المعاملات مقارنة بالكنترول وكانت منخفضة مع اللبتين 20 نانوجرام / مل عند مستوي معنوية (0.05%). أشارت النتائج إلي أن أعلى نسبة مئوية لمعدل الحمل كان مع اللبتين 20 نانوجرام / مل يليه الميلاتونين 10^{-3} M مقارنة بالكنترول (91.6، 75.0 و 66.6% علي التوالي). ونستخلص من هذه الدراسة أن إضافة أي من اللبتين والميلاتونين إلى مخفف الترس كإضافات حماية قد أدى إلى زيادة جودة السائل المنوي المجمد لطلائق الجاموس.