J. Agric. Sci. Mansoura Univ., 27 (10): 6655 - 6663, 2002.

THE INFLUENCE OF MELATONIN ON STORABILITY OF COOLED AND FROZEN BUFFALO SEMEN

El-Shamaá, I.S.
Animal Production Department, Faculty of Agriculture, Kafr El-Sheikh, Tanta University

ABSTRACT

Three semen ejaculates were collected weekly for eight successive weeks from three mature buffalo bulls to study the effect of melatonin on storability of buffalo bull semen. Only ejaculates with 70% of spermatozoa exhibiting progressive motility were included in the present study. The semen was extended by using Tris-egg yolk diluent to obtain 100 x 10^6 sperm per ml. Melatonin was added to diluted semen as 0.0, 10, 20 and 30 µg/100 x 10^6 sperm. Thereafter, diluted semen (control and treatments) was split into tow portions. The first part was stored at refrigerator (4°C) temperature for five days and examined daily for motility, live sperm percentage, sperm abnormality % as well as GOT, GPT release and fructose content in seminal plasma. The second portion was frozen in 0.25 ml straws after 4 h equilibration period at 5°C post dilution. Straws were stored in liquid nitrogen for 24 h before thawing in water bath at 37°C for 30 sec.

The results showed that the addition of 10 µg or 20 µg melatonin improved ($P < 0.05$) sperm motility, live sperm % and reduced percentage of sperm abnormality for 5 days of storage (at 4°C). The release of both GOT and GPT from spermatozoa into seminal plasma was significantly ($P < 0.05$) minimized in the presence of 20 µg melatonin compared with other treatments. Concerning fructose content in seminal plasma, addition of melatonin had a significant ($P < 0.05$) effect on conservation of energy in seminal plasma. The enzyme activities released to the seminal plasma were negatively correlated with sperm motility and positively correlated with sperm abnormality. The presence of 20 µg melatonin in the extender resulted in higher ($P < 0.05$) frozen-thawed motility compared to other treatments. It is concluded that buffalo semen could be successfully preserved for 5 days by addition of melatonin in Tris-extender at 4°C.or – 196°c

Keywords: Melatonin, buffalo semen, enzymes, frozen semen, storability.

INTRODUCTION

The success of semen storage depends on numerous factors which may be peculiar to each species and are optimized according to the type of semen to be preserved. Buffalo spermatozoa are more susceptible to hazards during storage of semen than cattle spermatozoa (Raizada *et al.,* 1990). These hazards can be minimized by methods that reduce or arrest the metabolism of spermatozoa and thereby prolonged their fertile live. Many attempts have been made to improve the basic buffers by inclusion of additives such as vitamins (Kolev, 1997), amino acids (Dhami and Sahni, 1993), chelating agents (Dhami *et al.*, 1994), enzymes (Dhami and Sahni, 1994), metabolic stimulants (El-Menoufy *et al.*, 1985) and hormones (Sansone *et al.,* 2000) to enhance sperm preservation and fertility (Maule, 1962). Several substances can interfere with microtubular function including colchicine and melatonin (Bornman *et al.,* 1989). In bull liquid semen,

extenders supplemented with different levels of melatonin gave a superior sperm motility percentages and reduced enzymes leakage from sperm cells preserved at 4°C compared to unsupplemented extenders (Anwar *et al.*, 1996).

Melatonin administration enhanced intact acrosome rate, reduced aspartate aminotransferase activity and post-thaw alkaline phosphatase release through rams spermatozoa (Keya *et al.*, 2001). Dhami and Kodagali (1990) and Dhami and Sahni (1994) utilized the GOT/GTP and lactic dehydrogenase (LDH) levels to assess the quality of spermatozoa after freeze-thawing procedures. They found a high negative correlation between fertility rates and leakage of these enzymes from sperm cells.

The present work was carried out to study the effect of supplementing buffalo semen with different levels of melatonin on sperm survival storage at refrigerator (4°C) temperature and frozen-thawed semen.

MATERIALS AND METHODS

Semen used throughout the experiment was collected by an artificial vagina from three mature buffalo bulls in regular use for breeding at Mehallet Mousa Experiment Station, Animal Production Institute, Ministry of Agriculture, for a period of eight weeks during summer season. Immediately after semen collection, ejaculate volume, sperm concentration (x 10^6/ml), percentage of sperm motility and percentages of live and abnormal spermatozoa were assessed (Table 1). Only samples with 70% of spermatozoa exhibiting progressive motility were included in the present study. The semen samples were diluted with Tris-egg yolk extender at 37°C to yield a concentration of 100 x 10^6 sperm/ml. The chemical components of the extender were: 3.61 g Tris [(hydroxymethyl) aminomethane], 1.89 g citric acid, 20 ml egg yolk, 5 ml glycerol, 0.25 g lincomycin, 0.005 g streptomycin and completed with distilled water to 100 ml). Melatonin (N-acetyl 5 methoxy tryptamine, product of Amoun Pharmaceutical Industries Company, Egypt) was added to the diluted semen as 0.0 µg (control sample), 10, 20 and 30 µg/100 x 10^6 sperm. Thereafter, diluted semen samples (treated and control) were split into two portions. The first part was stored in refrigerator (4°C) temperature and examined daily for five days to record sperm motility, live sperm % and sperm abnormalities %. After daily examination, samples were centrifuged at 3000 rpm for 20 minutes. The supernatant fluid were collected then kept at -20°C till used for determination of glutamic oxaloacetic transaminase (GOT, U/L), glutamic pyruvic transaminase (GPT, U/L) according to Reitman and Frankel (1957) and fructose content according to the technique adopted by Ashwell (1957) (Table 1a). The second diluted portion (control and treated samples) was packaged in mini straws (0.25 ml) which were sealed using polyvinyl powder. Three straws per each treatment were prepared, labeled and coded. Straws were placed horizontally in water bath at 37°C and transferred into the refrigerator to be cooled slowly to 5°C within one and half hour and left for four hours equilibration period before

freezing. For freezing, cooled straws were placed in freezing floating boat at 5-10 cm above LN_2 surface. Straws were exposed to LN_2 vapor for 10 min. then plunged directly into the LN_2 and stored for 24 hr. Frozen straws were thawed in water bath at 37°C for 30 sec. and percentages of progressive motility were recorded. The data were analyzed using the General linear Model of SPSS (1997).

Table (1): Initial characteristics of the buffalo semen used in this study.

Weeks	Ejaculate volume (ml)	Sperm motility %	Live sperm %	Abnormal sperm %	Sperm conc. x 10^9
1	2.0	75	92	6.5	1.065
2	1.5	80	88	8.5	2.073
3	3.0	75	90	7.5	1.890
4	2.3	72.5	91	6.5	2.280
5	2.3	75	93	8.0	1.640
6	1.5	75	86	12.0	1.417
7	1.5	72.5	91	8.0	1.118
8	1.5	75	92	7.5	2.115
$\bar{X} \pm$ SE	1.95±0.18	75±0.33	90.4±2.18	8.06±1.74	1.699±0.46

Table (1a): Initial values of GOT, GPT and fructose immediately after dilution.

Weeks	GOT (u/L)	GPT (U/L)	Fructose (mg/ml)
1	38.3	23.4	2.6
2	36.6	24.8	2.4
3	32.8	24.5	2.5
4	39.0	19.8	2.3
5	34.6	21.5	2.9
6	35.0	25.2	2.6
7	33.0	21.8	2.4
Overall mean ± SE	35.6±0.96	23.0±0.84	2.53±0.19

RESULTS AND DISCUSSION

Table 2 presents the effect of melatonin on the percentages of motile spermatozoa at various time of storage at refrigerator (4°C) temperature. It was observed that, samples containing melatonin had a significantly ($P < 0.05$) higher sperm motility than the control. In the meantime samples containing low levels of melatonin (10 or 20 µg) had significantly higher sperm motility % than the high level of melatonin (30 µg). From Table (2), it is apparent that, in all samples, sperm motility decreased by time increase. The rate of decline in motility with the increase in the time of storage was greater in the control when compared with treated samples. Adding 10 or 20 µg melatonin maintained sperm motility higher than 50% up to 96 hours of incubation.

These results are in agreement with those of Anwar *et al.* (1996) for bull liquid semen. They found that the effect of melatonin on sperm motility and survival may be attributed to the production of sufficient ATP and ATP-

ase in seminal plasma. Also, Guraya (1987) observed that melatonin increased c, AMP production in seminal plasma which stimulates sperm motility by direct action on the axoneme of the tail or by indirectly acting on cell membrane as a secondary messenger (Garbers and Kopf, 1980).

Table (2) shows the effect of melatonin on the percentage of live spermatozoa. Melatonin had a highest effect on the percentage of live spermatozoa after 48 hrs incubation. Beyond 120h live sperm percentage was maintained better with 20 µg melatonin compared with the other levels. These findings are consistent with the reports of Anwar *et al.* (1996) who found that melatonin reduced dead sperm % by the conservation of energy in seminal plasma.

Table (2): The effect of melatonin on the physical characteristics of buffalo semen stored at 4°C.

Storage time (hr)	Control	Melatonin concentration		
		10 µg	20 µg	30 µg
1. Sperm motility %				
24	$^{A}59.3 \pm 2.77^{e}$	$^{B}67.1 \pm 2.64^{d}$	$^{B}68.6 \pm 2.1^{d}$	$^{AB}65.0 \pm 2.67^{d}$
48	$^{A}53.6 \pm 2.82^{d}$	$^{B}62.9 \pm 2.4^{dc}$	$^{B}63.6 \pm 2.1^{cd}$	$^{AB}57.9 \pm 2.4^{cd}$
72	$^{A}42.9 \pm 2.4^{c}$	$^{B}57.1 \pm 2.14^{bc}$	$^{C}60.0 \pm 1.9^{bc}$	$^{B}51.4 \pm 2.37^{c}$
96	$^{A}32.1 \pm 1.49^{b}$	$^{C}50.7 \pm 2.02^{b}$	$^{C}54.3 \pm 2.02^{ab}$	$^{B}42.9 \pm 2.86^{b}$
120	$^{A}18.6 \pm 0.92^{a}$	$^{C}40.0 \pm 1.89^{a}$	$^{D}48.6 \pm 2.1^{a}$	$^{B}30.7 \pm 2.02^{a}$
Overall mean ± SE	41.3±2.68	55.6±1.92	59.02±1.48	49.6±2.3
2. Live sperm %				
24	$^{A}60.9 \pm 1.66$e	$^{C}86.4 \pm 1.31$e	$^{C}89.1 \pm 0.71$e	$^{B}83.0 \pm 0.62$e
48	$^{A}54.9 \pm 0.74^{d}$	$^{C}78.1 \pm 1.03^{d}$	$^{D}83.0 \pm 0.82^{d}$	$^{B}65.1 \pm 1.12^{d}$
72	$^{A}47.1 \pm 0.83^{c}$	$^{C}72.4 \pm 2.10^{c}$	$^{D}78.9 \pm 0.86^{c}$	$^{B}59.3 \pm 1.13^{c}$
96	$^{A}41.0 \pm 0.54^{b}$	$^{C}62.3 \pm 0.95^{b}$	$^{D}72.3 \pm 0.99^{b}$	$^{B}49.0 \pm 0.31^{b}$
120	$^{A}24.7 \pm 1.54^{a}$	$^{C}44.0 \pm 1.56^{a}$	$^{D}63.0 \pm 1.83^{a}$	$^{B}31.9 \pm 0.97^{a}$
Overall mean ± SE	45.7±2.19	68.7±2.58	77.3±1.58	57.7±2.93
3. Sperm abnormality %				
24	$^{C}13.3 \pm 0.42^{a}$	$^{B}10.7 \pm 0.35^{a}$	$^{A}8.6 \pm 0.57^{a}$	$^{B}10.4 \pm 0.57^{a}$
48	$^{C}15.3 \pm 0.56^{b}$	$^{B}12.3 \pm 0.28^{b}$	$^{A}10.1 \pm 0.45^{b}$	$^{B}12.9 \pm 0.26^{b}$
72	$^{D}18.1 \pm 0.55^{c}$	$^{B}13.6 \pm 0.20^{c}$	$^{A}11.4 \pm 0.36^{c}$	$^{C}14.9 \pm 0.26^{c}$
96	$^{D}24.1 \pm 0.67^{d}$	$^{B}14.6 \pm 0.20^{d}$	$^{A}12.7 \pm 0..35^{d}$	$^{C}16.1 \pm 0.40^{d}$
120	$^{D}30.9 \pm 0.76$e	$^{B}16.9 \pm 0.40$e	$^{a}13.3 \pm 0.28^{d}$	$^{C}18.9 \pm 0.50^{d}$
Overall mean ± SE	20.3±1.12	13.6±0.37	11.2±0.34	14.6±0.52

Treatment means within each row having different superscripts (A,B,C,...) are significantly different (P<0.05)

Storage time within each column having different superscripts (a,b,c,...) are significantly different (P< 0.05)

Sperm abnormality % was significantly ($P < 0.05$) lowered in the samples treated with melatonin (Table, 2).

The rate of increase in sperm abnormality % with the increase in the time of storage was lower in samples containing 10 or 20 µg melatonin than in other samples. This result is in agreement with data of Anwar *et al.* (1996) who found that 20 µg melatonin had a good protective effect for sperm. Luboshitzky *et al.* (2002) found that melatonin was a potent antioxidant and

efficient endogenous radical scavenger. Also, Poeggeler *et al.* (1993) reported that melatonin had antiaging effect on cells.

Extracellular activity of GOT during preservation of semen by cooling is presented in Table (3). The value of GOT release was significantly ($P < 0.05$) lowered with adding 20 or 10 µg followed by 30 µg melatonin, while, the highest GOT value was observed in control samples. This result might be due to the superiority of 20 or 10 µg melatonin in maintaining higher sperm motility and significantly prevented the enzyme leakage into the seminal plasma. The leakage of intracellular substances from spermatozoa into the extracellular fluid has been used as an indicator of sperm cell integrity (Dhami and Sahni, 1993).

Table (3): The effect of melatonin on GOT (U/L) release in seminal plasma stored at 4°C.

Storage time (hrs)	Control	Melatonin concentration		
		10 µg	20 µg	30 µg
24	$^{C}42.9\pm1.5^{a}$	$^{AB}33.4\pm0.84^{a}$	$^{A}31.7\pm0.42^{a}$	$^{B}35.9\pm0.26^{a}$
48	$^{C}50.0\pm1.22^{b}$	$^{B}37.6\pm0.84^{b}$	$^{A}33.1\pm0.34^{b}$	$^{B}38.0\pm0.31^{a}$
72	$^{D}57.0\pm1.05^{c}$	$^{B}39.1\pm0.96^{b}$	$^{A}34.7\pm0.42^{c}$	$^{C}42.3\pm0.75^{b}$
96	$^{D}63.9\pm1.26^{d}$	$^{B}44.1\pm1.14^{c}$	$^{A}39.9\pm0.46^{d}$	$^{C}48.3\pm0.94^{c}$
120	$^{C}72.3\pm1.13e$	$^{B}48.9\pm1.16^{d}$	$^{A}43.9\pm0.74e$	$^{B}50.9\pm1.34^{d}$
Overall mean ± SE	57.2±1.84	4.6±1.01	36.7±0.8	43.1±1.05

Treatment means within each row having different superscripts (A,B,C,...) are significantly different (P<0.05)

Storage time within each column having different superscripts (a,b,c,...) are significantly different (P< 0.05)

This increment of GOT release during semen preservation may be due to a possible liberation of an inhibitor which causes reduction in the enzyme activity (Brown *et al.* 1971).

Glutamic pyruvic transaminase (GPT, U/L) values varied according to the concentration of melatonin and storage times (Table, 4). It was noticed that, the minimum release of GPT enzyme was obtained with adding 20 µg melatonin followed by 10 µg and 30 µg melatonin, while the highest value was observed in control. The release of GOT and GPT to the extracellular fluid was correlated with sperm membrane premeability (Bower *et al.*, 1973) which could also be related to membrane disintegration and acrosome vesiculation (Azawi *et al.*, 1985). In the present study, the release of both GOT and GPT in the seminal plasma showed highly significant positive correlation ($r = 0.92$ and 0.89) with the percent of abnormal spermatozoa and were negatively correlated with the percent of motility ($r = -0.83$ and -0.80) and live sperm % ($r = -0.88$ and -0.85). The enzymes activities released to the extracellular fluid were negatively correlated ($r = -0.83$) with sperm motility and positively correlated ($r = 0.98$) with sperm with aged acrosomes (Azawi *et al.*, 1990).

The concentration of fructose in seminal plasma was significantly different ($P < 0.05$) between treated samples at various stages of time (Table, 5). At 120 h of preservation, samples containing either 10 or 20 µg melatonin has significant effect on fructose concentration, being 1.67 ± 0.06 and 1.79 ± 0.06 mg/ml, respectively. However, the samples containing 30 µg melatonin had higher fructose concentration compared to the control but differences

were not significant. This finding is in agreement with Anwar *et al.* (1996). In the present study, fructose content was positively correlated (r = 0.88) with live sperm % and negatively correlated (r = - 0.76) with sperm abnormality %.

Table (4): The effect of melatonin on GPT (U/L) release in seminal plasma stored at 4°C.

Storage time (hrs)	Control	Melatonin concentration		
		10 µg	20 µg	30 µg
24	$^{C}24.1 \pm 0.51^{a}$	$^{B}21.3 \pm 0.68^{a}$	$^{A}19.1 \pm 0.67^{a}$	$^{AB}20.7 \pm 0.68^{a}$
48	$^{C}26.1 \pm 0.71^{a}$	$^{B}22.3 \pm 0.71^{a}$	$^{A}19.9 \pm 0.59^{ab}$	$^{B}22.3 \pm 0.52^{a}$
72	$^{C}28.6 \pm 0.95^{b}$	$^{B}23.3 \pm 0.52^{ab}$	$^{A}21.0 \pm 0.79^{ab}$	$^{C}27.0 \pm 0.54^{b}$
96	$^{D}31.4 \pm 0.65^{c}$	$^{B}24.7 \pm 0.81^{b}$	$^{A}22.0 \pm 0.79^{bc}$	$^{C}27.4 \pm 0.65^{b}$
120	$^{C}34.0 \pm 0.62^{d}$	$^{B}26.9 \pm 0.83^{c}$	$^{A}23.7 \pm 0.68^{c}$	$^{B}28.3 \pm 0.68^{b}$
Overall mean ± SE	28.9±0.68	23.7±0.45	21.1±0.41	25.1±0.58

Treatment means within each row having different superscripts (A,B,C,...) are significantly different (P<0.05)
Storage time within each column having different superscripts (a,b,c,...) are significantly different (P< 0.05)

Post-thawed progressive motility was superior in the presence of 20 µg melatonin (44.3%) compared to 30 µg melatonin (31.4%) and control (38.9%, Table, 6). However, there were no significant differences in progressive frozen-thawed motility between 10 µg melatonin and both 20 µg melatonin and control.

Table (5): The effect of melatonin on fructose content (mg/ml) in seminal plasma stored at 4°C.

Storage time (hrs)	Control	Melatonin concentration		
		10 µg	20 µg	30 µg
24	$^{A}2.09 \pm 0.05^{e}$	$^{BC}2.34 \pm 0.06^{c}$	$^{C}2.51 \pm 0.09^{c}$	$^{AB}2.20 \pm 0.05^{e}$
48	$^{A}1.81 \pm 0.04^{d}$	$^{B}2.18 \pm 0.07^{bc}$	$^{C}2.44 \pm 0.09^{bc}$	$^{AB}1.99 \pm 0.04^{d}$
72	$^{A}1.66 \pm 0.03^{c}$	$^{B}2.03 \pm 0.06^{b}$	$^{C}2.23 \pm 0.09^{b}$	$^{A}1.82 \pm 0.04^{c}$
96	$^{A}1.52 \pm 0.02^{b}$	$^{C}1.81 \pm 0.05^{a}$	$^{D}1.99 \pm 0.05^{a}$	$^{B}1.68 \pm 0.04^{b}$
120	$^{A}1.37 \pm 0.02^{a}$	$^{B}1.67 \pm 0.06^{a}$	$^{B}1.79 \pm 0.06^{a}$	$^{A}1.49 \pm 0.02^{a}$
Overall mean ± SE	1.69±0.04	2.01±0.05	2.19±0.06	1.83±0.05

Treatment means within each row having different superscripts (A,B,C,...) are significantly different (P<0.05)
Storage time within each column having different superscripts (a,b,c,...) are significantly different (P< 0.05)

Table (6):The effect of melatonin on post-thawed progressive motility %.

Weeks	Control	Melatonin concentration		
		10 µg	20 µg	30 µg
1	40	50	40	30
2	35	40	50	35
3	40	45	40	30
4	45	35	50	35
5	35	40	45	30
6	35	45	45	30
7	40	40	40	30
Overall mean ± SE	B38.9±1.43	BC42.1±1.84	C44.3±1.7	A31.4±0.92

Treatment means within each row having different superscripts (A,B,C,...) are significant (P<0.05)

Only few studies have been done on the influence of melatonin on bovine bull spermatozoa *in vitro*, and research on this subject has been completely lacking for the sperm of buffalo bulls.

The present results revealed that melatonin can be used successfully for prolonged buffalo spermatozoa survival as well as increasing of sperm motility at refrigerator (4°C) temperature or post-thawed progressive motility. Also, it helps in minimizing release of both GOT and GPT from spermatozoa into seminal plasma and it helps in conservation of energy in seminal plasma during cooling.

REFERENCES

Anwar, M.M.; G.A. Megahed; Thorya, A. El-Deeb and H.S. Shehata (1996). The effect of melatonin on the bull liquid semen and enzymatic release in seminal plasma. Assiut Vet. Med. J., 35(69): 42-62.

Ashwell, G. (1957). Methods in Enzymol. Academic Press New York, p. 75.

Azawi, O.I.; M. Fathalla and A. Kamal-Uddin (1985). Effect of diluents glycerolization, freezing and thawing on the release of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) from bovine spermatozoa. Iraqi J. Vet. Med., 9: 54-62.

Azawi, O.I.; M.M. Znad and L.H. Al-Jarrah (1990). The correlation between the viability of bovine spermatozoa preserved at ambient temperature and the activity of transferases. Anim. Reprod. Sci., 22: 319-23.

Bornman, M.S.; J.M. Oesthuizen; H.C. Barnard; G.W. Schulenburg; D. Boomker and S. Reif (1989). Melatonin and sperm motility. Andrologia, 21(5): 483-85.

Bower, R.E.; B.G. Crabe; M.M. Pace and E.F. Graham (1973). Effect of dilution and glycerol on the release of glutamic oxaloacetic transaminase (GOT) from boar spermatozoa. J. Anim. Sci., 36: 319-24.

Brown, K.I.; B.G. Crabe; E.F. Graham and M.M. Pace (1971). Some factors affecting loss of intracellular enzyme from spermatozoa. Cryobiology, 8: 220-26.

Dhami, A.J. and S.B. Kodagali (1990). Freezability, enzyme leakage and fertility of buffalo spermatozoa in relation to the quality of semen ejaculates and extenders. Theriogenology, 34(5): 853-63.

Dhami, A.J. and K.L. Sahni (1993). Effect of extenders, additives and deep freezing on the leakage of transaminases from Murrah buffalo spermatozoa. Buffalo. J., 1: 55-64.

Dhami, A.J. and K.L. Sahni (1994). Effects of various cooling (from 30°C to 5°C), equilibration and diluent treatments on freezability, post-thaw thermoresistance, enzyme leakage and fertility of bubaline spermatozoa. Buffalo J., 2, 147-59.

Dhami, A.J.; V.R. Jani; G. Mohan and K.L. Sahni (1994). Effect of extenders and additives on freezability, post-thaw thermorestistance and fertility of frozen Murrah buffalo semen under tropical climate. Buffalo J.,1: 35-45.

El-Menoufy, A.A.; A.A. Seida; S.M. Fattouh and M.M. Ahmed (1985). Effect of caffeine on metabolic activity of ejaculated and epididymal spermatozoa of buffalo (*Bubalus bubalis*). Proc. 1st World Buffalo Cong., Cairo, Egypt, Vol. 1pp. 1078-1087.

Garbers, D.L. and G.S. Kopf (1980). The regulation of spermatozoa by calcium and cyclic nucleotides. In: Advances in Cyclic Nucleotide Research, 13, Greengard, P. and Robinson, G.A. (Eds.)., New York, Raven Press, pp. 251-306.

Guraya, S.S. (1987). "Biology of Spermatogenesis and Spermatozoa in Mammals". Berlin. Springier Verlag, pp. 286-302.

Keya, A.; M. Aksoy; N. Baspinar; C. Yildiz and M.B. Ataman (2001). Effect of melatonin implantation to sperm donor rams on post-thaw viability and acrosomal integrity of sperm cells in the breeding and non-breeding season. Reprod. Domest. Anim.; 36(3-4): 211-15.

Kolev, S.I. (1997). Effects of vitamins A, D, E on the motility and acrosomal integrity of cryopreserved buffalo bulls' spermatozoa. Proc. 5th World Buffalo Conf., Caserta, Italy Vol. 1, pp. 833-835.

Luboshitzky, R.; Z.O. Shen; R. Nave; S. Lavi and P. Lavie (2002). Melatonin administration alters semen quality in healthy men. J. Androl. 23(4): 572-78.

Maule, J.P. (1962). The semen of animals and artificial insemination Tech. Comm., 15. Commonwealth Agricultural Bureaux., Farnham Royal, England, pp. 420-30.

Poeggeler, B.; R.J. Reiter; D.X. Tan; L.D. Chen and L.C. Manchester (1993). "Melatonin hydroxyl radical mediated oxidative damage and aging: A hypothesis". J. Pineal Res., 14: 151-68.

Raitman, S. and S. Frankel (1957). Colorimetric determination of GOT and GPT activity. Am. J. Clin. Path., 28: 56.

Raizada, B.C.; A. Sattar and M.D. Pandey (1990). A comparative study of freezing buffalo semen in two diluters. In: Acharya, R.M.; Likeshwar, R.R.; Kumar, S. (Eds.).Recent Advances in Buffalo Res. Vol.3 pp.66-74.

Sansone, G.; M.J.F. Nastri and A. Fabbrocini (2000). Storage of buffalo (*Bubalus bubalis*) semen. Anim. Reprod. Sci., 62: 55-76.

SPSS (1997). SPSS Base 7.5 for Windows, User's Guide; SPSS Ind.

تأثير الميلاتونين على تخزين السائل المنوى المبرد والمجمد لطلائق الجاموس

ابراهيم سعد الشماع
قسم الانتاج الحيوانى – كلية الزراعة بكفرالشيخ – جامعة طنطا

استخدم فى هذا البحث ثلاثة قذفات من ثلاث طلائق جاموس ناضجة وذلك اسبوعيا ولمده ثمانى اسابيع متتاليه لدراسة تأثير الميلاتونين على تخزين السائل المنوى لطلائق الجاموس وقد استخدمت فقط القذفات التى تظهر حيواناتها المنوية حركة تقدميه مقدارها ٧٠%. وخفف السائل المنوى بمخفف التريس – صفار البيض للحصول على ١٠٠ × ١٠^٦ حيوان منوى لكل ١ مل. واضيف الميلاتونين للسائل المنوى المخفف بتركيزات صفر ، ١٠ ، ٢٠ ، ٣٠ ميكروجرام/١٠٠ مليون حيوان منوى. وتم تقسيم السائل المنوى المخفف الى قسمين. الجزء الاول حفظ على درجة حرارة الثلاجة (٤°م) لمدة خمسة أيام ولاختباره يوميا لتقدير النسبة المئوية للحيوانات المنوية المتحركة ، الحيه والشاذه وكذلك تحرر الـ GPT, GOT فى بلازما السائل المنوى وكذلك تم تقدير تركيز الفركتوز فى بلازما السائل المنوى. وتم تجميد الجزء الثانى من السائل المنوى المخفف فى انابيب بلاستيك بسعه ٠,٢٥ مللى بعد ٤ ساعات فترة تحضين وتوازن فى الثلاجة على درجة حراره ٤م° بعد التخفيف. بعد ٢٤ ساعة من التخزين فى النتروجين السائل تم تسييح الانابيب البلاستيك فى حمام مائى درجه حرارته ٣٧°م لمدة ٣٠ ثانية.

اظهرت النتائج أن اضافة ١٠ او ٢٠ ميكروجرام ميلاتونين حسن من حركه الحيوانات المنوية ، النسبة المئوية للحى وخفض النسبه المئوية للحيوانات المنوية الشاذة لمدة خمسة أيام من التخزين (على ٤°م). تحرر كل من انزيمى الـ GOT ، الـ GPT من الحيوانات المنويه الى بلازما السائل المنوي كان اقل ما يمكن (بمعنوية ٠,٠٥%) فى وجود ٢٠ ميكروجرام ميلاتونين بالمقارنة بالمعاملات الاخرى. ايضا اضافة الميلاتونين له تأثير معنوى على حفظ الطاقة فى بلازما السائل المنوى ويظهر ذلك من اختبار محتوى البلازما المنوية من الفركتوز. هناك علاقة ارتباطيه سالبه بين تحرر الانزيمات فى بلازما السائل المنوى وحركة الحيوانات المنوية وكذا علاقة ارتباطيه موجبه مع الحيوانات المنوية الشاذة. أعطى السائل المنوى المخفف المضاف اليه ٢٠ ميكروجرام ميلاتونين نسبه حيوية عالية بعد التسييح مقارنة بباقى المعاملات.

ويخلص البحث الى انه يمكن حفظ السائل المنوى لطلائق الجاموس بنجاح لمده خمسة أيام باضافة الميلاتونين إلى مخفف التريس المبرد على درجة حرارة الثلاجة(٤ °م).