CHANGES IN BLOOD PLASMA METABOLITES FOLLOWING MELATONIN IMPLANTATION IN BUFFALO BULLS DURING NON-BREEDING SEASON

Tamer Ramadan[1,*], Dharmendra Kumar[2] and Inderjeet Singh[2]

ABSTRACT

The purpose of this study was to investigate the effects of melatonin implants on biochemical metabolite composition and enzymatic properties of blood plasma of buffalo bulls in the non-breeding season. Ten Murrah buffalo bulls, divided into control and treated groups, each of five animals, were used during non-breeding season (July to September). Treated bulls were implanted with melatonin (18 mg of melatonin per 50 kg of body weight) for a period of two months. During non-breeding season, melatonin treatment resulted in increases ($P<0.05$) in total protein and creatinine of blood plasma, and decreases ($P<0.05$) in blood plasma high density lipoprotein-cholesterol (HDL), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities. In conclusion, melatonin treatment affected several metabolites in blood plasma of buffalo bulls during non-breeding season under tropical condition.

Keywords: *Bubalus bubalis*, buffaloes, melatonin, buffalo bulls, blood metabolites, non-breeding season

INTRODUCTION

Season is one of the most important factors that influence the reproduction and production potential of livestock. Seminal and biochemical parameters are significantly influenced by seasons. Thermal tolerance capacity of indigenous cattle is better than exotics as indicated by the good quality semen and lower sperm abnormalities (Rajoriya *et al*., 2013). Nearly in all the species, higher semen quality is obtained during spring season. Humid hot season is not desirable for the production of highly motile and fertile sperm. Buffalo bulls do better during winter and spring seasons, while zebu bulls produce sperm with better motility during spring and summer seasons (Kushwaha *et al*., 1955). The seasonal variation in freezability of buffalo bull semen indicated that the season affected significantly the post-thawing motility, with values being highest in ejaculates collected in winter and lowest in those collected in summer season (Sagdeo *et al*., 1991).

Melatonin is an indole derivative of endogenous compound secreted rhythmically from the pineal gland and influences the circadian clock and seasonal reproduction in mammals

[1]Animal Production Research Institute, Agricultural Research Center, Giza, Egypt, *E-mail: tamereweda1174@Yahoo.com

[2]Division of Animal Physiology and Reproduction, Indian Council of Agricultural Research, Central Institute for Research on Buffaloes, Haryana, India

(Reiter, 1991). Melatonin and its metabolites act as powerful antioxidants and antiapoptotic agents (Reiter *et al*., 1998). It has been reported that it has double the potency in action against peroxyl radicals as that of vitamin E (Pieri *et al*., 1994) and also it is more effective in scavenging the hydroxyl radicals than mannitol and reduced glutathione (Hardeland *et al*., 1993). Melatonin is associated with mitochondrial membrane potential (Acuna-Castroviejo *et al*., 2011), ATP production (Chen *et al*., 1994) and interaction with calmodulin (Tash *et al*., 1983). It stimulates cellular influx of Ca^{2+} into sperm cells (Delgadillo *et al*., 1994) and cyclic AMP (cAMP) stimulator (Yung *et al*., 1995) through which the melatonin stimulate the sperm to get higher motility and velocity.

Antioxidant effects of melatonin on seminal parameters were studied in rams, boars as well as bulls using refrigerated (unfrozen) semen bull samples (Ashrafi *et al*., 2013; Ramadan *et al*., 2019). This indolamine hormone interacts with the hypothalamus-pituitary axis to induce an increase in the pulsatile secretion of gonadotropin releasing hormone (GnRH), low levels of prolactin and augment luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone secretion (Lincoln and Clarke, 1997). It also, improves the quality of fresh and frozen semen in different breeds during the non-breeding season (Casao *et al*., 2010). However, studies to evaluate the effects of melatonin implantation on composition of blood plasma metabolites in the non-breeding season are scarce. The present study was performed to evaluate the concentrations of blood metabolic constituents in buffalo bulls in response to melatonin implants for preventing summer-induced decline in fertility of buffalo bulls.

MATERIALS AND METHODS

Location of study

The experiment was carried out on buffalo bulls during hot-humid season at the Central Institute for Research on Buffaloes (CIRB), Hisar, Haryana, India (latitude 29° 10′ N, 75° 46′ E). All procedures and experimental protocols were conducted in accordance with the guidelines laid down by Institute Animal Ethics Committee of the institute. Ejaculates were obtained from ten Murrah buffalo bulls aged 3 to 5 years, weighing 500 to 600 kg and kept at the bull shed of Semen Freezing Laboratory. Semen was collected twice a week using the artificial vagina technique during July to the first week of September, which is referred to as the hot-humid season with maximum ambient temperature and relative humidity ranging from 36 to 45°C and 30 to 80%, respectively. Bulls were kept in partly covered shed and partly open individual yard, fed individually on roughage and concentrate supplement according to body weight requirements (NRC, 20010) and water was freely accessible throughout the experimental period. Animals were disease-free, clinically normal and healthy.

Experimental design

The ten Murrah buffalo bulls were randomly allocated to melatonin non-implanted (control) and implanted (treated) groups (n = 5 each). For melatonin-treated group, animals were administered 2 x 4 mm absorbable melatonin implants (18 mg melatonin ⁄ implant, Regulin, CEVA Animal Health Limited, Chesham, Buckinghamshire, UK) at the base of the left ear using an implanter. Total implants inserted to each bull were calculated on the basis of their body weight (one implant ⁄ 50 kg) (Papachristoforou *et*

al., 2007). These implants are designed to induce high plasma melatonin for at least 60 days, although their functionality in animals can extend to more than 100 days (Forcada *et al*., 2002). Implants release melatonin continuously, without depleting the endogenous secretion of melatonin.

Blood collection

Blood samples were collected once a week throughout the experimental period via jugular vein-puncture into a heparinized vial. Plasma was separated immediately and kept in two aliquots at -20^{o}C until analysis. Samples from both groups were collected between 6 to 7 AM.

Parameters estimation

Blood plasma total protein, total albumin, glucose, total cholesterol, high density lipoproteins-cholesterol (HDL cholesterol), Transaminase activities [aspartate aminotransferase (AST) and alanine amino transferase (ALT)], Alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), Bilirubin total reagent (BIT), Creatinine (CRE) and Magnesium (Mg) were determined colorimetrically with auto analyzer using commercially available kits.

Statistical analysis

All data were tested for normality with the Shapiro–Wilk test from the UNIVARIATE procedure (SAS, 2004) and were found normally distributed (W≥0.90). Data were analyzed using the MIXED procedure of SAS (SAS, 2004). Treatment and weeks were used as fixed effects and individual bulls as random using the following model:

$$Y_{ijk} = \mu + T_i + W_j + (T_*W)_{ij} + e_{ijk}$$

Where Y_{ijk} = observed value of the dependent variable determined from a sample taken from each animal; μ = overall mean; T_i = fixed effect of the i th treatment (i = 1:2); w_j = fixed effect of the j th week (j = 1:8); $(T*W)_{ij}$ = first order interaction between treatments and days; e_{ijk} = The residual error. Differences among means within each class were tested using $LSD_{0.05}$.

RESULTS AND DISCUSSION

The results related to the effect of melatonin implantation amelioration on plasma metabolites of buffalo bulls during non-breeding season under tropical condition are presented in Table 1. Total protein was slightly higher in treated than control group. Many studies have shown that low protein contents of plasma were associated with poor sperm quality (Dhami and Kodagali, 1989; Taha *et al*., 2000). Plasma proteins are composed mainly of albumin and globulin in addition to small quantities of non-protein nitrogen, amino acids and peptides. These compounds make up the amphoteric property of plasma proteins and, thus, low protein contents in seminal plasma reduce its buffering capacity and in turn the semen quality (Dhami *et al*., 1994). In fact, the beneficial effects of plasma proteins in improving sperm motility reside in their content of albumin and other specific factors in seminal plasma, since epididymal spermatozoa lack progressive motility but acquire it upon the addition of either seminal plasma motility factor or albumin. The positive effect of melatonin administration on plasma total protein and albumin could be due to some of its antioxidant properties. Melatonin possesses the genomic property of regulating protein expression and enhancing activities of antioxidant enzymes

(Antolin *et al*., 1996). Almost similar values of albumin, glucose and cholesterol were observed in both experimental groups during non-breeding season without differences.

Among the climatic factors, photoperiod is the main environmental cue influencing seasonality. Though it is documented that seasonality of reproduction in seasonal breeders is mediated through melatonin, a neuro-hormone secreted by the pineal gland (Borghese *et al*., 1994), we assessed the melatonin concentrations in buffalo bulls during non-breeding season not to be different between treated bulls and control (Ramadan *et al*., 2019). Melatonin acts as a neuroendocrine mediator of the photoperiod and, in humans and other diurnal mammals, its production occurs during the dark phase. Therefore, day length alters the pattern of melatonin secretion, which is controlled by an internal clock, named suprachiasmatic nuclei, located on the hypothalamus (Carlomagno *et al*., 2011). Melatonin is capable of acting on the hypothalamic–pituitary axis to influence the release of gonadotropins, which in turn, regulate gonadal function. It influences testosterone production from the Leydig cells and sperm quality by its antiapoptotic and scavenging properties (Rocha *et al*., 2015).

Lower ($P<0.05$) plasma HDL cholesterol was found in treated buffalo bulls (34.97 mg/dL) compared to control (38.98 mg/dL). Melatonin acts as an antioxidant by preventing efflux of cholesterol from the sperm membrane and consequently prohibits premature capacitation and acrosomal reactions (Ashrafi *et al*., 2013). Along with phospholipids, cholesterol is necessary for cell physical integrity and ensures fluidity of the cell membrane (Srivastava *et al*., 2013). It plays a special role in the sperm membrane function because its release from the sperm membrane initiates the key step in the process of capacitation and acrosome reaction that is crucial for fertilization. It has been reported recently that melatonin prevents *in vitro* sperm capacitation and apoptotic like changes (Casao *et al*., 2010), which demonstrates a direct action of this hormone on spermatozoa. The effect of melatonin in preventing apoptosis like changes may be related to its antioxidant and free radical scavenging activities that increases fertility rate (Casao *et al*., 2010).

As indicated previously, seminal plasma lipids play significant role in the membrane structure of spermatozoa. Also, Kelso *et al*. (1997) reported that reduction in sperm concentration and motility were associated with a decrease in seminal plasma content of lipids as well as sperm aging (poor semen quality). Cholesterol is a precursor for the synthesis of male sex hormones. Guyton, (1991) reported that male sex hormones (androgens) increase blood cholesterol.

The levels of AST and ALT enzymes actives in plasma are very important for sperm metabolism and function (Brooks, 1990). They provide energy for survival, motility and fertility of spermatozoa. Transaminase activities in semen are good indicators of its quality because they measure the stability of sperm membrane (Corteel, 1980). Thus, the increased percentage of abnormal spermatozoa in the preservation causes high concentration of transaminase enzymes in the extracellular fluid due to sperm membrane damage and ease of enzymes leakage from spermatozoa (Gundogan, 2006). In the present study, AST level was low ($P<0.05$) in blood plasma of the melatonin treated bulls as it was utilized in stabilizing the membrane integrity of acrosome, plasma, mitochondria and flagella of the sperm (Leon *et al*., 2005; Lopez *et al*., 2009). Also, the increased concentrations of AST and ALT in semen

Table 1. Effect of melatonin implantation on blood plasma metabolites of buffalo bulls during non-breeding season (least square means ± SEM).

Parameters	Treatment	
	Control	Melatonin
Total protein (g/dl)	9.07±0.12[b]	9.59±0.11[a]
Albumin (g/dl)	3.53±0.02	3.64±0.05
Glucose (mg/dl)	68.93±1.79	70.42±0.94
Cholesterol (mg/dl)	65.70±0.91	64.25±0.99
HDL cholesterol (mg/dl)	38.98±0.53[a]	34.97±0.59[b]
AST (IU/L)	153.83±3.35[a]	139.59±2.46[b]
ALT (IU/L)	57.58±1.38[a]	54.72±0.91[b]
ALP (U/I)	147.19±6.41	145.85±5.08
GGT (U/I)	19.21±0.46	20.65±0.46
BIT (mg/dl)	0.16±0.01	0.15±0.01
Creatinine (mg/dl)	1.89±0.03[b]	2.25±0.05[a]
Magnesium (mg/dl)	4.61±0.04	4.57±0.07

HDL: High density lipoproteins-cholesterol, AST: aspartate aminotransferase,
ALT: alanine aminotransferase, ALP: alkaline phosphatase,
GGT: γ-glutamyltransferase, BIT: bilirubin total reagent,
[a-b] Within a row, means without a common superscript differ (P<0.05).

were reported to be associated with increased percentage of dead spermatozoa due to sperm membrane damage and ease of enzymes leakage from spermatozoa (Pace and Graham, 1970). Kaya *et al*. (2000) showed that melatonin administration did not exert any significant effect on seminal plasma AST concentration. Polakoski *et al*. (1976) stated that seminal plasma enzymes, which can be measured at higher concentrations in the first portion of ejaculate, such as aminotransferase are probably of prostatic origin. Moreover, Ramadan *et al*. (2009) reported reductions in AST seminal plasma levels in Damascus male goats during out-of-breeding season more than during the rest of the year. This confirms the antioxidative effect of melatonin on goat spermatozoa since the concentration of transaminase enzymes in semen is a good indicator of its high quality as it measures sperm membrane stability (Pace and Graham, 1970). In addition, the results of the present study showed that the effects of melatonin on the biochemical parameters are dose-dependent and could be attributed to the stimulatory effects of melatonin on the activity of enzymes involved in antioxidant defence and in accordance with those of Casao *et al*. (2010); Ashrafi *et al*. (2013).

The molecular events/mechanisms that determine the fertilizing potential of a semen sample are not well understood yet. Melatonin receptors have been evidenced in epididymis, prostate and testis (Izzo *et al*., 2010) suggesting these cells as targets of the pineal hormone. The concentration of molecules related to different pathways involved in the acquisition of sperm quality is altered in the plasma of rams treated with melatonin. The high concentration of these metabolites in conjunction with the better sperm competence suggests that these molecules, acting in synergy to melatonin, promote energy metabolism and antioxidant defense, improving the quality of the sperm. Therefore, they can be involved in determining the fertilization potential of the sperm during the non-breeding season, acting mainly in the antioxidant defense.

To the best of our knowledge, the known effects of melatonin implantation on buffalo semen quality were scarce during non-breeding season under tropical condition. The beneficial effect of melatonin-implantation on semen characteristics and seminal plasma constituents of buffalo bulls in the present study was similar to those reported using extender supplemented with melatonin in buffalo bulls (El-Raey *et al*., 2014; Husna *et al*., 2017) and cow bulls (Ashrafi *et al*., 2013).

In conclusion, melatonin treatment during non-breeding season showed significant increases in the blood metabolites TP and CRE and reduction of HDL cholesterol, AST and ALT during the non-breeding season under tropical condition.

ACKNOWLEDGMENTS

This research was funded by a research grant awarded by the CV Raman International Fellowship for African Researchers under Post-Doctoral Fellowship, New Delhi, India.

REFERENCES

Acuna-Castroviejo, D., L.C. Lopez, G. Escames, A. Lopez, J.A. Garcia and R.J. Reiter. 2011. Melatonin-mitochondria interplay in health and disease. *Curr. Top. Med. Chem*., **11**(2): 221-240. DOI: 10.2174/156802611794863517

Antolin, I., C. Rodriguez and R.M. Sainz. 1996. Neurohormone melatonin prevents cell

damage: Effect on gene expression for antioxidant enzymes. *Federation of American Societies for Experimental Biology*, **10**(8): 882-890. DOI: 10.1096/fasebj.10.8.8666165

Ashrafi, I., H. Kohram and F.F. Ardabili. 2013. Antioxidative effects of melatonin on kinetics, microscopic and oxidative parameters of cryopreserved bull spermatozoa. *Anim. Reprod. Sci*., **139**(1): 25-30. DOI: 10.1016/j.anireprosci.2013.03.016

Borghese, A., V.L. Barile, G.M. Terzano, A.M. Pilla and A. Parmeggani. 1994. Melatonin trend during seasons in heifers and buffalo cows. *In Proceedings of 4th World Buffalo Congress, Sao Paulo*, Brazil. **3**: 528-530.

Brooks, D.E. 1990. Biochemistry of the male accessory glands. p. 569-690. *In Marshall's Physiology of Reproduction*, *G.E. Lamming*, 4th ed., Churchill Livingstone, Edinburgh, Scotland.

Carlomagno, G., M. Nordio, T.T. Chiu and V. Unfer. 2011. Contribution of myo-inositol and melatonin to human reproduction. *European J. Obstetr. and Gynec. and Reprod. Biol*., **159**(2): 267-272. DOI: 10.1016/j.ejogrb.2011.07.038

Casao, A., I. Cebrian, M.E. Asumpcao, R. Perez-Pe, J.A. Abecia, F. Forcada, J.A. Cebrián-Pérez and T. Muiño-Blanco. 2010. Seasonal variations of melatonin in ram seminal plasma are correlated to those of testosterone and antioxidant enzymes. *Reprod. Biol. Endocrin*., **8**(1): 59. DOI: 10.1186/1477-7827-8-59

Chen, L., P. Kumar, R.J. Reiter, D.X. Tan, L.C. Manchester, J.P. Chambers, B. Poeggeler and S. Saarela. 1994. Melatonin prevents the suppression of cardiac Ca^{+2} stimulated ATPase activity induced by Alloxan. *Am. J. Physiol. Endoc. M*., **267**(1): E57-E62. DOI: 10.1152/ajpendo.1994.267.1.E57

Corteel, J.M. 1980. Effects du plasma s´eminalsur la survie et la fertilit´e des spermatozoids conserv´es *in vitro. Reprod. Nutr. Dev*., **20**(4): 1111-1123. Available on: https://hal.archives-ouvertes.fr/hal-00897713/document

Delgadillo, L.H., F.A. Tay and G.B. King. 1994. Effect of melatonin on microtubule assembly depend on hormone concentration: Role of melatonin as a calmodulin antagonist. *J. Pineal Res*., **17**(2): 55-62. DOI: 10.1111/j.1600-079x.1994.tb00114.x

Dhami, A.J., G.M. Klasahni and R.P. Tripathi. 1994. Comparative evaluation of initially static and motile semen ejaculates from Friesian and Murrah buffalo bulls for physico-morphological, biochemical, enzymatic and mineral constituents of seminal plasma. *Indian J. Anim. Sci*., **64**: 926-932.

Dhami, A.J. and S.B. Kodagali. 1989. Comparative analysis of physicobiochemical attributes in static and motile semen ejaculates from Surti buffalo bulls. *Indian J. Anim. Sci*., **59**(3): 344-347.

El-Raey, M., M.R. Badr, Z.M. Rawash and G.M. Darwish. 2014. Evidences for the role of melatonin as a protective additive during buffalo semen freezing. *American Journal of Animal and Veterinary Sciences*, **9**(4): 252-262. DOI: 10.3844/ajavsp.2014.252.262

Forcada, F., J.A. Abecia, O. Zuniga and J.M. Lozano. 2002. Variation in the ability of melatonin implants inserted at two different times after the winter solstice to restore reproductive activity in reduced seasonality ewes. *Aust. J. Agr. Res*., **53**(2):1 67-173.

DOI: 10.1071/AR00172

Gundogan, M. 2006. Some reproductive parameters and seminal plasma constituents in relation to season in Akkaraman and Awassi rams. *Turk. J. Vet. Anim. Sci*., **30**(1): 95-100.

Guyton, A.C. 1991. *Textbook of Medical Physiology*, 8th ed. W.B. Saunders Company, Philadelphia, Pennsylvania, USA. p. 754-764.

Hardeland, R., R.J. Reiter, B. Poeggeler and D.X. Tan. 1993. The significance of the metabolism of the neurohormone melatonin: Antioxidative protection and formation of bioactive substances. *Neurosci. Biobehav. R*., **17**(3): 347-357. DOI: 10.1016/s0149-7634(05)80016-8

Husna, A., M.S. Ansari, B.A. Rakha, R. Ejaz, N. Ullah and S. Akhter. 2017. Melatonin supplementation in extender enhances the post thaw quality of buffalo bull spermatozoa. *Pak. J. Zool*., **49**(1): 163-167. DOI: 10.17582/journal.pjz/2017.49.1.163.167

Izzo, G., A. Francesco, D. Ferrara, M.R. Campitiello, I. Serino, S. Minucci and M. d'Istria. 2010. Expression of melatonin (MT1, MT2) and melatonin-related receptors in the adult rat testes and during development. *Zygote*, **18**(3): 257-264. DOI: 10.1017/S0967199409990293

Kaya, A., N. Baspinar, C. Yildiz, F. Kurtoglu, M.B. Ataman and S. Haliloglu. 2000. Influence of melatonin implantation on sperm quality, biochemical composition of the seminal plasma and plasma testosterone levels in rams. *Rev. Med. Vet-Toulouse*, **151**(12): 1143-1146.

Kelso, K.A., A. Redpath, R.C. Noble and B.K. Speake. 1997. Lipid and antioxidant changes in spermatozoa and seminal plasma throughout the reproductive period of bulls. *J. Reprod. Fertil*., **109**(1): 1-6. DOI: 10.1530/jrf.0.1090001

Kushwaha, N.S., D.P. Mukherji and P. Bhattacharya. 1955. Seasonal variation in reaction time and semen quality of buffalo bulls. *International Journal of Veterinary Sciences and Animal Husbandry*, **25**: 317-328.

León, J., D. Acuña-Castroviejo, G. Escames, D.X. Tan and R.J. Reiter. 2005. Melatonin mitigates mitochondrial malfunction. *J. Pineal Res*., **38**(1): 1-9. DOI: 10.1111/j.1600-079X.2004.00181.x

Lincoln, G.A. and I.J. Clarke. 1997. Refractoriness to a static melatonin signal develops in the pituitary gland for the control of prolactin secretion in the ram. *Biol. Reprod*., **57**(2): 460-467. DOI: 10.1095/biolreprod57.2.460

López, A., J.A. García, G. Escames, C. Venegas, F. Ortiz, L.C. López and D. Acuña-Castroviejo. 2009. Melatonin protects the mitochondria from oxidative damage reducing oxygen consumption, membrane potential, and superoxide anion production. *J. Pineal Res*., **46**(2): 188-198. DOI: 10.1111/j.1600-079X.2008.00647.x

NRC. 2001. *Nutrient Requirement of Dairy Cattle*, 7th ed. National Academy of Science, Washington, D.C., USA.

Pace, M.M. and E.F. Graham. 1970. The release of GOT from bovine spermatozoa as a test method of assaying semen quality and fertility. *J. Biol. Reparat*., **3**: 140-146.

Papachristoforou, C., A. Koumas and C. Photiou. 2007. Initiation of the breeding season in ewe lambs and goat kids with melatonin implants. *Small Ruminant Res*., **73**(1-3): 122-126. DOI: 10.1016/j.

smallrumres.2006.12.004

Pieri, C., M. Marra, F. Moroni, R. Recchioni and F. Marcheselli. 1994. Melatonin: A peroxyl radical scavenger more effective than vitamin E. *Life Sci*., **55**(15): 271-276. DOI: 10.1016/0024-3205(94)00666-0

Polakoski, K.L., F.N. Syner and L.J.D. Zaneveld. 1976. Biochemistry of human seminal plasma. *In* Hafez, E.S.E. (ed.) *Human Semen and Fertility Regulation in Men*. The C.V. Mosby Company, St. Louis, Mo, USA. p. 140.

Rajoriya, J., J.K. Prasad, S. Ghosh and M. Singh. 2013. Effects of seasons on enzymatic changes and cholesterol efflux in relation to freezability in Tharparkar bull semen. *Asian Pacific Journal of Reproduction*, **2**(4): 280-288. DOI: 10.1016/S2305-0500(13)60163-3

Ramadan, T.A., D. Kumar, S.S. Ghuman and I. Singh. 2019. Melatonin-improved buffalo semen quality during non-breeding season under tropical condition. *Domest. Anim. Endocrin*., **68**: 119-125. DOI: 10.1016/j.domaniend.2019.01.010

Ramadan, T.A., T.A. Taha, M.A. Samak and A. Hassan. 2009. Effectiveness of exposure to longday followed by melatonin treatment on biochemical and enzymatic properties of seminal plasma of Damascus male goats during breeding and out-of-breeding seasons. *Egyptian Journal of Basic and Applied Physiology*, **8**(2): 415-432.

Reiter, R.J. 1991. Pineal melatonin: Cell biology of its synthesis and of its physiological interactions. *Endocrine Reviews*, **12**(2): 151-180. DOI: 10.1210/edrv-12-2-151

Reiter, R.J., D.X. Tan, S.J. Kim and W. Qi. 1998. Melatonin as a pharmacological agent against damage to lipids and DNA. *In Proceedings of the Western Pharmacology Society*, **41**: 229-236.

Rocha, C.S., L. Rato, A.D. Martins, M.G. Alves and P.F. Oliveira. 2015. Melatonin and male reproductive health: Relevance of darkness and antioxidant properties. *Curr. Mol. Med.*, **15**(4): 299-311. DOI: 10.2174/1566524015666150505155530

Sagdeo, L.R., A.B. Chitins, S.N. Deshmukh and A.S. Kaikini. 1991. Seasonal variations in relation to freezability of semen of Jersey and crossbred bulls with varying level of exotic inheritance. *Indian J. Anim. Reprod*., **12**(2): 117-121.

SAS. 2004. *SAS user's Guide: Statistics, Version* 8th ed. SAS Inst., Inc., Cary, North Carolina, USA.

Srivastava, N., S.K. Srivastava, S.K. Ghosh, A. Kumar, P. Perumal and A. Jerome. 2013. Acrosome membrane integrity and cryocapacitation are related to cholesterol content of bull spermatozoa. *Asian Pacific Journal of Reproduction*, **2**(2): 126-131. DOI: 10.1016/S2305-0500(13)60132-3

Taha, T.A., E.I. Abdel-Gawad and M.A. Ayoub. 2000. Monthly variations in some reproductive parameters of Barki and Awassi rams throughout 1 year under subtropical conditions. 2. Biochemical and enzymatic propertics of seminal plasma. *Anim. Sci*., **71**(2): 325-332. DOI: 10.1017/S1357729800055168

Tash, J.S. and A.R. Means. 1983. Cyclic adenosine 3', 5' monosphate, calcium and protein phosphorylation in flagellar motility. *Biol. Reprod*., **28**(1): 75-104. DOI: 10.1095/biolreprod28.1.75

Yung, L.Y., S.T. Tsim and Y.H. Wong. 1995. Stimulation of cAMP accumulation by the

cloned Xenopus melatonin receptor through G1 AND G2 proteins. *FEBS Letters*, **372**: 99-102. DOI: 10.1016/0014-5793(95)00963-A