Buffalo Bulletin (January-March 2024) Vol.43 No.1
https://doi.org/10.56825/bufbu.2024.4313563

ASSESSMENT OF OXIDATIVE STRESS PARAMETERS IN SUMMER ANESTROUS BUFFALOES EXHIBITING DIFFERENTIAL FERTILITY FOLLOWING MELATONIN IMPLANTS TREATMENT

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Received: 01 May 2020
Accepted: 21 March 2024

ABSTRACT

For evaluating the impact of melatonin implants treatment during non-breeding season to ameliorate oxidative stress, 132 anestrous buffaloes were subcutaneously inserted with 2x4 mm absorbable slow-release melatonin implants (18 mg/50 kg b wt) at the base of left ear and 60 buffaloes were used as control. Ovarian ultrasonography and jugular vein blood sampling were carried out at 7-day interval till day 35 post-treatment or till ovulation, whichever was earlier. Control and implanted buffaloes were subjected to artificial insemination (AI) at overt or induced estrus followed by pregnancy diagnosis at day 90 post-AI. Erythrocytic lipid peroxidation (LPO) values were reduced ($P<0.05$) in implanted buffaloes from day 21 post-treatment onwards when compared to their pre-treatment and Control group values. However, the concentrations of erythrocytic glutathione peroxidase (GPx), glutathione reductase and superoxide dismutase (SOD) were invariably higher ($P<0.05$) following treatment as compared to their pre-treatment and Control group values. The buffaloes ovulating in Control or Treatment group revealed higher ($P<0.05$) erythrocytic GPx in the Latter group. Also, between pregnant counterparts of Control and Treatment group, the Latter group buffaloes exhibited low ($P<0.05$) erythrocytic LPO, and high ($P<0.05$) erythrocytic GPx, SOD and catalase. It can be concluded that melatonin implants treatment was successful for mitigating the oxidative stress in summer anestrous buffaloes, and the status of oxidative stress parameters following treatment was better in buffaloes that ovulated or conceived subsequently.

Keywords: *Bubalus bubalis*, buffaloes, anestrus, antioxidant, fertility, melatonin

INTRODUCTION

Seasonal suppression of fertility in buffaloes is influenced by exogenous (photoperiod, climate, nutrition, management) and endogenous (hormones, genotype) factors (D'Occhio *et al*., 2020). In summer season, buffalo species is prone to detrimental consequences of ionizing radiations due to their dark skin coat. In fact, summer season induced oxidative stress subsequently leads to decline in fertility (Guérin *et al*., 2001). High oxidative stress results in increased levels of

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reactive oxygen species (ROS) free radicals such as superoxides, hydroxyl radicals and hydrogen peroxide (Rodriguez *et al*., 2004). In fact, the ovarian follicles of anestrous buffaloes had higher concentrations of follicular ROS (Jan *et al*., 2014). Further, ROS triggers the progressive destruction of polyunsaturated fatty acids (lipid peroxidation, LPO), ultimately leading to membrane destruction (Reina and Martinez, 2018). This oxidative stress induced damage to cellular components can be controlled by various antioxidant defense mechanisms including exogenous antioxidants (Rock *et al*., 1996). Melatonin (N-acetyl-5-methoxytryptamine) is synthesized by the pineal gland during the dark phase of photoperiod and plays a major role in a variety of physiological functions, including regulation of circadian rhythms associated with visual, reproductive, cerebrovascular, neuroendocrine and neuroimmune actions (Reiter, 1993; Reiter, 1998). Melatonin is a strong free radical scavenger and can reverse the adverse effects of oxidative stress by reducing LPO (Rodriguez *et al*., 2004; Reina and Martinez, 2018). Moreover, melatonin stimulates gene expression of antioxidative enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (Rodriguez *et al*., 2004). Therefore, melatonin can be used as an important medication for increasing conception rate in summer season by protecting the sperms, oocytes and embryos cells from the free radical damage and apoptosis (Medrano *et al*., 2017).

Melatonin treatment of anestrous buffaloes during summer season was able to reduce oxidative stress (Kumar *et al*., 2014; Lochan *et al*., 2020). *In vitro* developmental competence of oocytes was improved by increasing their antioxidant capacity, as the high lipid content makes buffalo oocytes/embryos highly sensitive to oxidative damages (Boni *et al*., 1992). Moreover, melatonin has an important role in the maintenance of pregnancy due to a specific genotype of MTNR1A gene which was more sensitive to melatonin treatment that favored pregnancy in buffaloes during summer season (Pandey *et al*., 2019).

Nevertheless, the literature is not available regarding the differential antioxidant defense mechanisms in melatonin-treated buffaloes that subsequently ovulated or conceived as compared to their non-ovulatory or non-conceiving counterparts. Hence, we investigated the oxidative stress status in summer anestrous buffaloes exhibiting differential fertility status following melatonin implants treatment.

MATERIALS AND METHODS

The present study was conducted on anestrous buffaloes (mean age: 51.10±3.12 months, mean body weight: 425.19±29.89 kg) of various smallholder dairy farmers near to Veterinary University, Ludhiana (latitude: 30°56'N, longitude: 75°52'E), India. The summer hot humid season (April to August) with maximum ambient temperatures ranging from 36 to 45°C and relative humidity 30 to 80%) was chosen for the experiment. For the confirmation of anestrus during the 60-day period before the start of experiment, all the buffaloes were subjected to three observations per day for the detection of overt estrus and the buffaloes failing to show overt estrus were included in the study. Using an ultrasound scanner, all the buffaloes were subjected to gynaecological examination before inclusion in the study, and the buffaloes diagnosed with any pathological condition of the reproductive tract were not included.

Experimental design

Anestrous buffaloes were randomly allocated to non-implanted control (n=60) or implanted Treatment (n=132) group. Using an implanter, 2×4 mm size absorbable slow releasing melatonin implants (18 mg melatonin/implant, Regulin, CEVA Animal Health Limited, France) were administered in the Treatment group buffaloes at base of left ear one implant/50 kg (Ghuman *et al*., 2010). The principle of insertion of these implants was to induce high plasma melatonin for at least 60 days, on the other hand their functionality can extend up to 100 days in ewes (Forcada *et al*., 2002).

Ultrasonography

Ovarian ultrasonography was carried out in each buffalo starting from the day of implants-insertion till day 35 or till ovulation, whichever was earlier, at weekly interval using B-mode ultrasound scanner equipped with linear-array rectal transducer (Sonosite Vet M Turbo, USA). Similar procedures were followed for non-implanted controls. The occurrence of ovulation in a buffalo was determined on the basis of presence (dominant non-ovulatory follicle) or absence (ovulatory follicle) of dominant follicle on subsequent examination(s). Ovulation was verified based upon the subsequent emergence of a corpus luteum (CL) on a site previously occupied by the disappeared dominant follicle (Ghuman *et al*., 2010). During the study period, the control group had 43 non-ovulatory and 17 ovulatory buffaloes, whereas treatment group had 59 non-ovulatory and 73 ovulatory buffaloes.

Blood sampling and oxidative stress parameters

Blood samples in a heparinized vial were collected from the jugular vein after each ultrasonography. Haemoglobin content (g%) was immediately estimated using haemocytometer. For the preparation of erythrocytic lysate, blood samples were centrifuged at 3000 rpm for 15 minutes. Plasma was separated and kept in two aliquots at -20°C for analysis. The remaining haematocrit was washed thrice with tris-citrate buffer (5 mM Tris HCl, pH 7.4; 120 mM NaCl; 1 mM EDTA) by centrifugation at 3000 rpm for 10 minutes. By mixing the haematocrit with distilled water, erythrocytic lysate was prepared and stored at -20°C for further analysis. Using standard protocols, these aliquots were used for estimation of oxidative stress parameters *viz*. Lipid Peroxidation (MDA formed µ mole/g Hb), Glutathione peroxidase (µ mole NADPH oxidised/g Hb/min), Glutathione reductase (µ mole/g Hb) and Superoxide dismutase (unit/g Hb) and Catalase (units/g Hb).

Artificial insemination (AI) and conception rate

During the study period, all the buffaloes were subjected to three observations per day for the detection of overt estrus. Buffaloes exhibiting overt estrus during were inseminated using frozen semen. Buffalo, failing to show overt estrus were subjected to fixed time AI protocol (FTAI) on day 35 of study period. This protocol involved administration (i/m) of gonadotropin releasing hormone (GnRH, 0.02 mg) on day 0 and 9, and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 500µg cloprostenol sodium) on day 7. In addition, buffaloes were inserted per-vaginally a controlled internal drug release (CIDR, 1.38 g progesterone) device for 7 days (day 0 to 7). On day 10 of protocol, the fixed time AI was done using frozen semen. Per-rectal pregnancy diagnosis of all the inseminated buffaloes was carried out on day 90 post-insemination. By the end of study period, the control group had 39 non-pregnant and 21 pregnant

buffaloes, whereas treatment group had 56 non-pregnant and 76 pregnant buffaloes.

Statistical analysis

Numerical data are represented as mean±SEM, and differences were considered to be significant at P<0.05. Two samples of Student's t-test were employed on data of oxidative stress parameters of various days of study period for assessing the differences in: (a) pre-treatment *vs.* post-treatment, (b) control *vs*. treatment group, and (c) ovulatory *vs.* non-ovulatory and conceiving *vs.* non-conceiving buffaloes within and between groups. Statistical analyses were performed using MINITAB release 13.2 statistical software (Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSIONS

The buffaloes with melatonin implants treatment (d0) exhibited a decrease (d2 1 to d35; P<0.05) in erythrocytic LPO. Moreover, the implanted buffaloes had lower erythrocytic LPO compared to their control counterparts (d28, P<0.05; Table 1). The detailed comparison of LPO data with respect to occurrence of ovulation or subsequent conception revealed low concentrations in conceived buffaloes of Implanted group compared to their non-implanted counterparts (d35, P<0.05; Table 4). This suggested melatonin-induced beneficial impact of reduced LPO which lead to subsequent conception in buffaloes. The mammalian gametes high high levels of polyunsaturated fatty acids are highly susceptible to oxidation of the membrane lipids by partially reduced oxygen molecules, such as superoxide, hydrogen peroxide and hydroxyl radicals. This results in adverse consequences such as compromised fertility (Gavella *et al*., 1996; Asadpour *et al*., 2011). In fact, a significant improvement in viability, nuclear morphology, and plasma membrane intactness of gametes was observed with addition of exogenous melatonin (El-Battawy and Sckalcki, 2015).

During post-melatonin implant study period of 35 days, the antioxidant activity was improved as revealed by elevated concentrations of erythrocytic GPx (P<0.05; Table 1) and SOD (P<0.05; Table 2) in implanted buffaloes. Furthermore, compared to non-implanted counterparts, the implanted buffaloes had elevated erythrocytic GR (d14 and d21, P<0.05; Table 1) and SOD (d14-28, P<0.05; Table 2) activity. Superoxide dismutase and GPx prevent the accumulation of free radicals and ROS by inhibiting their formation (van Zoeren-Grobben *et al*., 1996). Therefore, the increased activities of erythrocytic SOD in implanted buffaloes could be attributed to melatonin-induced upregulation of this enzyme in an attempt to mitigate superoxide radical challenge. Moreover, antioxidant enzymes (GPx, GR, SOD, and catalase) also exhibited an increasing trend in buffaloes till day 35 following melatonin-treatment (Singh *et al*., 2016). Melatonin treatment in lactating buffaloes during out-of-breeding season increased SOD activity and improved the reproductive performance (Ramadan *et al*., 2016).

The detailed comparison of antioxidant enzyme data during post-implant period with respect to occurrence of ovulation or subsequent conception revealed that buffaloes exhibiting ovulation had increased activity of erythrocytic GPx (d28 and d35), and the buffaloes conceiving subsequently had increased activity of erythrocytic GPx (d7), SOD (d28 and d35) and catalase (d28) compared to their non-implanted counterparts (P<0.05; Table 3 and 4). These results were indicative

Table 1. Status of oxidative stress (LPO - Lipid Peroxidation, MDA formed µ mole/g Hb; GPx - Glutathione Peroxidase, µ mole NADPH oxidised/g Hb/min; GR - Glutathione Reductase, µ mole/g Hb) in erythrocytes of control (n=60) and melatonin-implanted (n=132) buffaloes. Day 0 = Insertion of melatonin implants.

Day	Control			Melatonin-implanted		
	LPO	GPx	GR	LPO	GPx	GR
0	1.91±0.18	4.78±0.30	0.033±0.007	1.96±0.09[a]	5.00±0.21[a]	0.029±0.002
7	1.89±0.17	5.27±0.37	0.031±0.003	1.84±0.08	5.93±0.22	0.032±0.002
14	1.81±0.16	5.24±0.34	0.023±0.002[c]	1.71±0.07	6.19±0.22[b]	0.036±0.002[d]
21	1.97±0.15	5.85±0.40	0.024±0.002[c]	1.60±0.06[b]	6.07±0.28[b]	0.032±0.002[d]
28	1.80±0.13[c]	5.62±0.35	0.026±0.002	1.42±0.06[b,d]	6.11±0.28[b]	0.029±0.003
35	1.75±0.15	5.56±0.44	0.025±0.002	1.38±0.07[b]	6.72±0.32[b]	0.027±0.002

[a] *vs* [b]P<0.05, within a column; [c] *vs* [d]P<0.05, within a row of a parameter.

Table 2. Status of oxidative stress (SOD - Superoxide Dismutase, unit/g Hb; Catalase, units/g Hb) in erythrocytes of control (n=60) and melatonin-implanted (n=132) buffaloes. Day 0 = Insertion of melatonin implants.

Day	Control		Melatonin-implanted	
	SOD	Catalase	SOD	Catalase
0	7.27±0.40	0.0050±0.0004	7.32±0.24[a]	0.0049±0.0002
7	7.50±0.42	0.0054±0.0004	8.52±0.30[b]	0.0052±0.0002
14	7.10±0.31[c]	0.0047±0.0003	8.51±0.31[b,d]	0.0053±0.0002
21	6.86±0.30[c]	0.0045±0.0003	8.04±0.32[d]	0.0053±0.0002
28	7.03±0.34[c]	0.0050±0.0003	8.59±0.34[b,d]	0.0056±0.0003
35	7.32±0.44	0.0053±0.0004	8.64±0.40[b]	0.0057±0.0003

[a] *vs* [b]P<0.05, within a column; [c] *vs* [d]P<0.05, within a row of a parameter.

Table 3. Post-treatment ovulation status (NO, Non-ovulated; O, Ovulated) versus oxidative stress status (LPO - Lipid Peroxidation, MDA formed µ mole/g Hb; GPx - Glutathione Peroxidase, µ mole NADPH oxidised/g Hb/min; GR - Glutathione Reductase, µ mole/g Hb; SOD - Superoxide Dismutase, unit/g Hb; Catalase, units/g Hb) in erythrocytes of control (C; NO=43, O=17) and melatonin-implanted (MI; NO=59, O=73) buffaloes. Day 0 = Insertion of melatonin implants

Parameter	Gp		Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
LPO	C	NO	1.76±0.21	1.81±0.22	1.89±0.17	2.07±0.14	1.85±0.13	1.81±0.16
		O	2.11±0.30	2.06±0.28	1.41±0.47	1.00±0.45	1.21±0.65	1.24±0.59
	MI	NO	1.95±0.12[a]	1.79±0.12	1.59±0.10	1.63±0.08	1.42±0.08[b]	1.38±0.08[b]
		O	1.96±0.12[a]	1.87±0.11	1.80±0.09	1.57±0.09	1.42±0.09[b]	1.37±0.12[b]
GPx	C	NO	4.85±0.36	5.40±0.42	5.23±0.36	5.83±0.33	5.89±0.33	5.91±0.41
		O	4.68±0.55[a]	5.02±0.75	5.30±1.00	6.12±1.84	4.48±0.30[b,c]	4.79±0.54[b,c]
	MI	NO	5.43±0.33	5.64±0.33	5.91±0.33	5.87±0.40	6.00±0.38	6.56±0.45
		O	4.72±0.26[a]	6.14±0.29[b]	6.40±0.30[b]	6.33±0.38[b]	6.28±0.40[b,d]	7.00±0.38[b,d]
GR	C	NO	0.039±0.011	0.026±0.002	0.023±0.002[c]	0.024±0.002	0.025±0.002	0.025±0.002
		O	0.024±0.003	0.040±0.006	0.022±0.002[e]	0.030±0.002	0.033±0.001	0.030±0.002
	MI	NO	0.027±0.003	0.032±0.003	0.035±0.004[d]	0.031±0.002	0.033±0.002	0.031±0.002
		O	0.031±0.002	0.033±0.003	0.036±0.003[f]	0.028±0.003	0.032±0.003	0.027±0.003
SOD	C	NO	7.37±0.53	7.11±0.46	7.00±0.36	6.80±0.32	7.03±0.37	7.29±0.49
		O	7.11±0.63	8.25±0.82	7.61±0.41	7.48±1.00	7.01±0.84	7.54±0.86
	MI	NO	7.55±0.49	7.84±0.40	7.79±0.47	7.48±0.41	8.34±0.48	8.68±0.55
		O	7.17±0.22[a]	9.01±0.41[b]	9.05±0.40[b]	8.71±0.49[b]	8.94±0.47[b]	8.57±0.56
Catalase	C	NO	0.0049±0.0004	0.0057±0.0004	0.0048±0.0003	0.0045±0.0003	0.0051±0.0003	0.0054±0.0004
		O	0.0053±0.0006	0.0050±0.0006	0.0043±0.0009	0.0045±0.0019	0.0045±0.0020	0.0049±0.0018
	MI	NO	0.0049±0.0003	0.0051±0.0003	0.0052±0.0004	0.0051±0.0003	0.0055±0.0004	0.0057±0.0004
		O	0.0049±0.0003	0.0052±0.0003	0.0054±0.0003	0.0055±0.0003	0.0058±0.0004	0.0058±0.0006

[a] *vs* [b] $P<0.05$, within a row; [c] *vs* [d], [e] *vs* [f] $P<0.05$, within a column of a parameter.

Table 4. Post-treatment conception status (P, Pregnant; NP, Non-pregnant) versus oxidative stress status (LPO - Lipid Peroxidation, MDA formed µ mole/g Hb; GPx - Glutathione Peroxidase, µ mole NADPH oxidised/g Hb/min; GR - Glutathione Reductase, µ mole/g Hb; SOD - Superoxide Dismutase, unit/g Hb; Catalase, units/g Hb) in erythrocytes of control (C; NP=39, P=21) and melatonin-implanted (MI; NP=56, P=76) buffaloes. Day 0 = Insertion of melatonin implants.

Parameter	Gp		Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
LPO	C	NP	1.75±0.19	1.79±0.21	1.78±0.18	1.95±0.15	1.73±0.15	1.68±0.19
		P	2.20±0.35	2.06±0.30	1.88±0.34	2.01±0.33	1.96±0.26	2.03±0.08[c]
	MI	NP	1.96±0.14[a]	1.75±0.13	1.48±0.09	1.52±0.09	1.31±0.08[b]	1.37±0.08[b]
		P	1.96±0.11[a]	1.90±0.10	1.88±0.10	1.65±0.08	1.50±0.08[b]	1.38±0.12[b,d]
GPx	C	NP	5.21±0.37	5.74±0.44	5.38±0.44	5.67±0.55	5.52±0.46	5.31±0.47
		P	3.92±0.43	4.47±0.64[c]	4.97±0.53	6.20±0.56	5.85±0.55	6.43±1.12
	MI	NP	4.92±0.33	5.47±0.35	5.83±0.35	6.06±0.48	6.12±0.44	6.54±0.46
		P	5.08±0.25	6.29±0.27[d]	6.45±0.29	6.08±0.35	6.11±0.36	6.93±0.44
GR	C	NP	0.032±0.010	0.030±0.004	0.022±0.002[c]	0.023±0.002	0.026±0.002	0.025±0.002
		P	0.036±0.006	0.032±0.004	0.024±0.003[e]	0.027±0.004	0.026±0.005	0.027±0.005
	MI	NP	0.028±0.002	0.032±0.003	0.034±0.003[d]	0.031±0.003	0.033±0.002	0.032±0.002
		P	0.030±0.003	0.033±0.003	0.037±0.003[f]	0.028±0.003	0.032±0.003	0.027±0.002
SOD	C	NP	7.17±0.43	6.62±0.49	7.27±0.32	6.94±0.27	7.07±0.41	7.46±0.54
		P	7.45±0.85	8.98±0.57	6.73±0.69	6.70±0.75	6.94±0.64[c]	6.81±0.68[c]
	MI	NP	7.45±0.33	7.96±0.34	8.08±0.37	7.74±0.45	7.95±0.45	8.06±0.47
		P	7.20±0.34[a]	8.96±0.45[b]	8.82±0.46[b]	8.25±0.45	9.08±0.49[b,d]	9.33±0.67[b,d]
Catalase	C	NP	0.0051±0.0004	0.0055±0.0004	0.0047±0.0004	0.0043±0.0004	0.0052±0.0004	0.0055±0.0005
		P	0.0049±0.0007	0.0053±0.0007	0.0047±0.0006	0.0047±0.0008	0.0046±0.0001[c]	0.0048±0.0002
	MI	NP	0.0048±0.0003	0.0053±0.0003	0.0053±0.0003	0.0053±0.0004	0.0056±0.0004	0.0059±0.0005
		P	0.0049±0.0003	0.0051±0.0003	0.0053±0.0003	0.0053±0.0003	0.0057±0.0004[d]	0.0055±0.0004

[a] *vs* [b] $P<0.05$, within a row; [c] *vs* [d], [e] *vs* [f] $P<0.05$, within a column of a parameter.

of the valuable impact of melatonin-induced antioxidant enzyme activity on reproductive axis. It was suggested that SOD plays an important role in the maintenance of luteal function, possibly that SOD acts protectively against superoxide radicals to stimulate progesterone production by the CL (Laloraya *et al*., 1998). In fact, SOD and catalase activities in CL had pattern similar to plasma progesterone as these enzymes had 6 to 8-fold increase from day 6 to 16 of the estrous cycle and then decreased during luteal regression (Rapoport *et al*., 1998). Furthermore, melatonin supplementation to serum free maturation media improved the fertilization rate of buffalo oocytes (Nagina *et al*., 2016). Melatonin also has a critical role in blastocyst implantation in a number of different mammalian species, including sheep, ferrets, horses, hamsters and rats (Reiter, 1998).

In conclusion, the present study indicated that slow-release melatonin implants treatment in anestrous buffaloes during summer season can be efficiently utilized to mitigate the oxidative stress with subsequent beneficial impact on fertility status.

REFERENCES

Asadpour, R., R. Jafari and H. Tayefi-Nasrabadi. 2011. Effect of various levels of catalase antioxidant in semen extenders on lipid peroxidation and semen quality after the freeze-thawing bull semen. *Vet. Res. Forum*, **2**(4): 218-221.

Boni, R., L. Santella, B. Dale, S. Roviello, R. Di Palo and V. Barbieri. 1992. Maturazione *in vitro* di oociti buffalini: Indagine ultrastrutturale. *Acta Med. Vet*., **38**: 153-161.

D'Occhio, M.J., S.S. Ghuman, G. Neglia, G. Valle, P.S. Baruselli, L. Zicarelli, J.A. Visintin, M. Sarkar and G. Campanile. 2020. Exogenous and endogenous factors in seasonality of reproduction in buffalo: A review. *Theriogenology*, **150**: 186-192. DOI: 10.1016/j.theriogenology.2020.01.044

El-Battawy, K.A. and M. Sckalcki. 2015. Seminal Profile, antioxidant enzymes activities and levels of testosterone in Tyrolean mountain rams after melatonin implantation. *Middle East Journal of Applied*, **5**(4): 1232-1238. Available on: https://www.curresweb.com/mejas/mejas/2015/1232-1238.pdf

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. Agri. Res*., **53**(2): 167-173. DOI: 10.1071/AR00172

Gavella, M., V. Lipovac, M. Vucic and B. Rocic. 1996. Relationship of sperm superoxide dismutase-like activity with other sperm-specific enzymes and experimentally induced lipid peroxidation in infertile men. *Andrologia*, **28**(4): 223-229. DOI: 10.1111/j.1439-0272.1996.tb02787.x

Ghuman, S.P.S., J. Singh, M. Honparkhe, D. Dadarwal, G.S. Dhaliwal and A.K. Jain. 2010. Induction of ovulation of ovulatory size non-ovulatory follicles and initiation of ovarian cyclicity in summer anoestrous buffalo heifers (*Bubalus bubalis)* using melatonin implants. *Reprod. Domest. Anim*., **45**(4): 600-607. DOI: 10.1111/j.1439-0531.2008.01310.x

Guérin, P., M.S. El and Y. Ménézo. 2001. Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and

its surroundings. *Human Reprod. Update*, **7**(2): 175. DOI: 10.1093/humupd/7.2.175

Jan, M.H., G.K. Das, F.A. Khan, J. Singh, S.T. Bashir, S. Khan, J.K. Prasad, S. Mehrotra, M.C. Pathak, G. Singh and M. Sarkar. 2014. Evaluation of follicular oxidant-antioxidant balance and oxidative damage during reproductive acyclicity in water buffalo (*Bubalus bubalis*). *Asian Pacific Journal of Reproduction*, **3**(1): 35-40. DOI: 10.1016/S2305-0500(13)60182-7

Kumar, A., S. Mehrotra, G. Singh, K. Narayanan, G.K. Das, Y.K. Soni, M. Singh, A.S. Mahla, N. Srivastava and M.R. Verma. 2015. Sustained delivery of exogenous melatonin influences biomarkers of oxidative stress and total antioxidant capacity in summer-stressed anestrous water buffalo (*Bubalus bubalis*). *Theriogenology*, **83**: 1402-1407. DOI: 10.1016/j.theriogenology.2014.12.023

Laloraya, M., P.G. Kumar and M.M. Laloraya. 1988. Changes in the levels of superoxide anion radical and superoxide dismutase during the estrous cycle of Rattus norvegicus and induction of superoxide dismutase in rat ovary by lutropin. *Biochem. Bioph. Res. Co*., **157**(1): 1146-1153. DOI: 10.1016/s0006-291x(88)80025-1

Lochan, S., M. Honparkhe, R.S. Cheema, A. Kumar, S.P.S. Ghuman and P.S. Brar. 2020. Ameliorating postpartum reproductive cyclicity using Exogenous melatonin implant in water buffalo (*Bubalus bubalis*). *Indian J. Anim. Sci*., **90**(2), 181-184. DOI: 10.56093/ijans.v90i2.98772

Medrano, A., C.F. Contreras, F. Herrera and A. Alcantar-Rodriguez. 2017. Melatonin as an antioxidant preserving sperm from domestic animals. *Asian Pacific Journal of Reproduction*, **6**(6): 241-246. DOI: 10.4103/2305-0500.217317

Nagina, G., A. Asima, U. Nemat and A. Shamim. 2016. Effect of melatonin on maturation capacity and fertilization of *Nili-Ravi* buffalo (*Bubalus bubalis*) oocytes. *Open Veterinary Journal*, **6**(2): 128-134. DOI: 10.4314/ovj.v6i2.9

Pandey, A.K., P. Gunwant, N. Soni, Kavita, S. Kumar, A. Kumar, A. Magotra, I. Singh, J.B. Phogat, R.K. Sharma, Y. Bangar, S.P.S. Ghuman and S.S. Sahu. 2019. Genotype of MTNR1A gene regulates the conception rate following melatonin treatment in water buffalo. *Theriogenology*, **128**: 1-7. DOI: 10.1016/j.theriogenology.2019.01.018

Ramadan, T.A., R.K. Sharma, S.K. Phulia, A.K. Balhara, S.S. Ghuman and I. Singh. 2016. Manipulation of reproductive performance of lactating buffaloes using melatonin and controlled internal drug release device treatment during out-of-breeding season under tropical conditions. *Theriogenology*, **86**(4): 1048-1053. DOI: 10.1016/j.theriogenology.2016.03.034

Rapoport, R., D. Sklan, D. Wolfenson, A. Shaham-Albalancy and I. Hanukoglu. 1998. Antioxidant capacity is correlated with steroidogenic status of the corpus luteum during the bovine estrous cycle. *Biochem. Biophys. Acta*, **1380**(1): 133-140. DOI: 10.1016/S0304-4165(97)00136-0

Reina, M. and A. Martinez. 2018. A new free radical scavenging cascade involving melatonin and three of its metabolites (3OHM, AFMK and AMK). *Comput. Theor. Chem*., **1123**: 111-118. DOI: 10.1016/j.comptc.2017.11.017

Reiter, R.J. 1993. The melatonin rhythm: both a clock and a calendar. *Experientia*, **49**(8):

654-664. DOI: 10.1007/BF01923947

Reiter, R.J. 1998. Melatonin and human reproduction. *Ann. Med*., **30**(1): 103-108. DOI: 10.3109/07853899808999391

Rock, C.L., R.A. Jacob and P.E. Bowen. 1996. Update on the biological characteristics of the antioxidant micronutrients: vitamin C, vitamin E, and the carotenoids. *J. Am. Diet. Assoc*., **96**(7): 693-702. DOI: 10.1016/S0002-8223(96)00190-3

Rodriguez, C., J.C. Mayo, R.M. Sainz, I. Antolin, F. Herrera, V. Martin and R.J. Reiter. 2004. Regulation of antioxidant enzymes: A significant role for melatonin. *J. Pineal Res*., **36**(1): 1-9. DOI: 10.1046/j.1600-079x.2003.00092.x

Singh, B., S.P.S. Ghuman, R.S. Cheema and A.K. Bansal. 2016. Melatonin implant induces estrus and alleviates oxidative stress in summer anestrus buffalo. *Indian J. Anim. Reprod*., **37**(2): 28-32.

Van Zoeren-Grobben, D., J.H.N. Lindeman, E. Houdkamp, R. Bland, J. Schrijver and H.M. Berger. 1994. Postnatal changes in plasma chain-breaking antioxidants in healthy preterm infants fed formula and/or human milk. *Am. J. Clin. Nutr*., **60**(6): 900-906. DOI: 10.1093/ajcn/60.6.900