MELATONIN IMPLANT INDUCES ESTRUS AND ALLEVIATES OXIDATIVE STRESS IN SUMMER ANESTRUS BUFFALO

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ABSTRACT

Summer anestrus buffalo were divided into control (n=29) and treatment group (n=41). Treated buffalo were subcutaneously inserted melatonin implants @18mg/50kg body wt. Estrus signs were observed till 35 days post-treatment and estrus induction rate (%) was recorded. Blood samples were collected at weekly intervals, 7 days prior to insertion of implant until day 35 after insertion of implant. Estrus induction rate was higher (χ^2=3.19, p<0.05) in melatonin-implanted buffalo (65.8%) as compared to control (41.4%). Lipid peroxide (LPO) concentrations reduced (p<0.05) in melatonin-implanted buffalo from day 28 onwards as compared to values in pre-treatment period and controls. Antioxidant enzymes (Glutathione Peroxidase, Glutathione Reductase, Superoxide dismutase and Catalase) showed an increasing trend following melatonin-treatment and remained elevated until day 35 (p<0.05); however, no such phenomenon was recorded in control buffalo. In conclusion, the present study demonstrated the effectiveness of melatonin for inducing estrus and alleviation of oxidative stress in summer anestrus buffalo.

Keywords: Buffalo**,** Melatonin implant, Oxidative stress, Summer anestrus

INTRODUCTION

Domestic buffalo have a tendency to breed seasonally and show a reproductive pattern that is closely related to the environmental and climatic conditions (Das and Khan, 2010). This reproductive seasonality results into cessation of ovarian cyclic activity and subsequently, fertility as day-light hours increase, a condition popularly known as summer anestrus. During summer, only a small proportion of Indian buffalo (26%) exhibit estrus. Furthermore, a higher incidence of abbreviated duration of estrus and silent estrus are observed during summer (70% in April *vs.* 10% in December; Ghuman, 2014). During summer anestrus, although one or more ovulatory size follicles develop, ovulation fails to occurs due to endocrine insufficiencies (Ghuman *et al.,* 2010). The regulation of gonadotropin releasing hormone (GnRH)/luteinizing hormone (LH) secretion by melatonin hormone may be central to the seasonal regulation of ovarian cyclicity in buffalo (Zicarelli, 1997). The Mediterranean buffalo showing seasonal reproductive trend had highest night-time plasma melatonin in winter (more dark hours) and lowest in summer (less dark hours; Parmeggiani *et al.,* 1994). Melatonin is known to alleviate the impact of strong negative feedback of estrogen on GnRH axis during non-breeding season in ewe (Karsch *et al.,* 1984). Treatment with sustained release melatonin implant mimics the effect of short days by lengthening the daily presence of melatonin. This rise in circulating melatonin, through its action both on hypothalamus and pituitary, is in turn responsible for at least 10-fold increase in plasma concentrations of GnRH and gonadotrophins, thus leading to follicular growth and ovulation (Misztal *et al.,* 2002). In summer anestrus buffalo heifers, using melatonin implants, 100% ovulatory estrus induction response was observed (Ghuman *et al.,* 2010).

Melatonin possesses the ability to neutralize reactive oxygen species, reduces lipid peroxide

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concentrations as well as DNA damage, thereby improving the viability of germ cells (Nakano *et al.,* 2012). Melatonin, being a powerful free radical scavenger, has been hypothesized to have properties essential for establishment of successful pregnancy and prevention of spontaneous abortion (Tamura *et al.,* 2008). The protective effects of melatonin are due to its ability to reduce NO formation, scavenge peroxynitrite associated oxidants and posseses indirect antioxidant activity (Korkmaz *et al.,* 2011). Thus, the present study was designed to investigate the impact of melatonin implant on estrus induction response and alleviation of oxidative stress in summer anestrus buffalo.

MATERIALS AND METHODS

The study was conducted on 70 anestrus buffalo (300-600 kg body weight) selected from private rural dairy farms of Punjab. All the buffalo were daily fed 10-15 kg green fodder, 8-10 kg wheat straw, 2-3 kg concentrate, 50-60 g mineral mixture and had free access to drinking water.

Using an implanter, treatment group buffalo (n=41) were subcutaneously administered 2x4 mm absorbable melatonin implants (18 mg melatonin/ implant, Regulin®, CEVA Animal Health Limited, Chesham, Buckinghamshire, UK) at the base of left ear. The number of implants to be inserted in each buffalo was calculated on the basis of body weight (one implant/50 kg; Ghuman *et al.,* 2010). These implants were designed to release plasma melatonin for at least 60 days. Another, 29 buffalo without any implant treatment served as control during the study period. In each buffalo, blood samples were collected from the jugular vein at weekly interval starting a week before the day of implant insertion. The sampling protocol lasted till the onset of overt estrus and artificial insemination or maximum till day 35 post-treatment. During the experiment period, buffalo were subjected to three observations per day for the detection of overt estrus.

Haemoglobin content was estimated using haemocytometer and expressed as g% was recorded. For the preparation of RBC lysate, blood was collected from jugular vein in a heparinised vial and centrifuged at 3000 rpm for 15 min. Plasma was separated immediately 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 min. By mixing the haematocrit with distilled water, RBC lysate was prepared, stored at -20˚C for further analysis. Using standard published protocols, these aliquots were used for estimation of oxidative stress parameters viz. Lipid peroxide (LPO; µmole/g Hb), Superoxide dismutase (SOD; unit/g Hb), Glutathione Peroxidase (GPx; IU/g Hb/ min), Glutathione Reductase (GR; IU/g Hb/min) and Catalase (units/litre).

The differences between groups were examined by Duncan's Multiple range (DMRT) test followed by Least Significant Difference (LSD) test using SPSS version 16.0 for Windows.

RESULTS AND DISCUSSION

Estrus induction response was higher (χ^2=3.19; $p<0.05$) in melatonin-implanted buffalo (65.8%) as compared to control (41.4%) during summer season. However, the estrus induction rate in melatonin-treated buffalo observed in the present study was lower than the previous reports (100% by Ghuman *et al.,* 2010 and 90% by Kumar *et al.,* 2015). This could be due to better estrus detection adopted in organised dairy farms as compared to the current study which was carried out at the field level in buffalo of marginal and small farmers.

In the present study, LPO concentrations remained uniform in the control group, whereas, in the implanted buffalo, a decreasing trend ($p<0.05$) was observed from day 21 post-treatment onwards as compared to pre-treatment concentrations. Between groups, lower ($p<0.05$) LPO concentrations were recorded from day 21 onwards in the implanted buffalo (Figure 1). In control

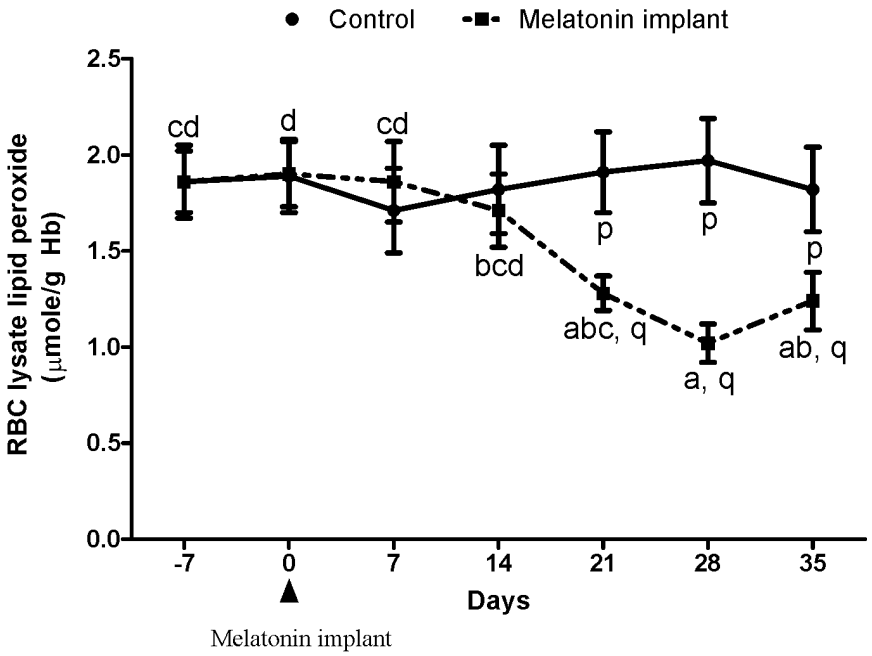

Figure 1: Lipid peroxide concentrations (Mean±SE) in control and melatonin-implanted buffalo. [a] *vs.* [b] *vs.* [c]; [p] *vs.* [q]**p<0.05**

buffalo, GPx concentrations remained uniform during the study period, whereas in treatment group, GPx increased ($p<0.05$) and reached a peak level between day 7-21 after melatonin implant and returned to pre-treatment levels at day 35. In addition, the treatment group displayed higher ($p<0.05$) concentration of GPx as compared to control at days 14 and 21 (Table 1). With regard to GR, melatonin-treated buffalo achieved highest ($p<0.05$) concentration on day 7 and 14 post-treatment as compared to control group and the pre-treatment levels (Table 1). Similarly, SOD increased ($p<0.05$) at day 7 and 14 post-treatment and returned to pre-treatment level after day 21. Melatonin-treated buffalo had higher ($p<0.05$) SOD as compared to control between day 7 and 28 post-treatment (Table). Furthermore, the catalase increased ($p<0.05$) in melatonin-treated group from day 14 onwards and remained consistently elevated up to day 35 (Table 1).

In the present study, melatonin treatment reduced oxidative stress in RBC lysate by decreasing lipid peroxidation and increasing the activity of antioxidants in summer anestrus buffalo. The results of the present study are consistent with a recent report in summer anestrus buffalo, which also documented the potential of exogenous melatonin to augment the antioxidative capacity of summer anoestrus buffalo (Kumar *et al.,* 2015). The capacity of melatonin to reduce membrane damage under condition of high oxidative stress indicates that its mechanism of action relates to its antioxidant activity by directly scavenging free radicals and inhibiting the membrane peroxidation (Reiter *et al.,* 2001). The physiochemical properties of melatonin provide high diffusion ability through subcellular compartments (Reiter *et al.,* 2001). *In vitro* and *in vivo* studies have also reported the protective role of melatonin against detrimental oxidants (Tamura *et al.,* 2008 and Bonnefont-Rousselot *et al.,* 2011). Melatonin stimulates the activity of several enzymes related to antioxidative defence system (Pieri *et al.,* 1995), most likely through its binding with membrane receptors or by nuclear- or cytosolic-binding sites (Tomas-Zapio *et al.,* 2005). Another study found that the inhibitory

Table 1: Effect of melatonin implant treatment glutathione peroxidise (GPx; IU/g Hb/min)**, glutathione reductase** (GR; IU/g Hb/min)**, superoxide dismutase** (SOD; unit/g Hb) **and catalase** (U/l) **in buffalo RBC hemolysate** (Mean±SE)

Control					
Day	n	**GPx**	**GR**	**SOD**	**Catalase**
-7	29	4.73±0.23	0.031±0.003	6.91±0.40	4.59±0.28^{b}
0	29	4.62±0.29	0.034±0.007	7.27±0.41	4.92±0.25^{b}
7	26	4.60±0.38	0.032±0.003	7.30±0.51	4.62±0.33^{b}
14	19	4.51±0.37	0.027±0.002	6.82±0.46	4.24±0.22^{ab}
21	19	4.81±0.40	0.031±0.002	6.49±0.36	4.14±0.18^{ab}
28	18	4.67±0.29	0.045±0.013	6.94±0.44	3.58±0.13^{a}
35	17	4.81±0.23	0.027±0.002	7.07±0.49	3.59±0.20^{a}
Melatonin implant					
-7	41	4.20±0.20^{ab}	0.035±0.003^{ab}	7.30±0.29^{a}	4.72±0.31
0	41	3.96±0.22^{a}	0.039±0.003^{abc}	7.28±0.35^{a}	5.06±0.34
7	33	5.71±0.40^{c*}	0.051±0.004^{cd*}	10.14±0.61^{b*}	5.50±0.38
14	27	5.67±0.43^{c*}	0.057±0.004^{d*}	9.52±0.66^{b*}	5.29±0.29*
21	20	5.45±0.40^{c}	0.046±0.005^{bcd*}	9.21±0.79^{ab*}	5.16±0.31*
28	16	5.19±0.51^{bc}	0.045±0.006^{bcd}	9.21±1.03^{ab*}	5.13±0.24*
35	14	4.75±0.33^{abc}	0.027±0.002^{a}	9.11±1.13^{ab}	5.23±0.33*

*p<0.05, between treatment groups; $^{a\ vs.\ b\ vs.\ c}$ p<0.05, values with different superscript differ within a column of a group

effect of H_2O_2 on oocytes maturation was significantly blocked by simultaneous addition of melatonin (Tamura *et al.,* 2008). A model proposed that single-electron exchange is the basis for interactions of melatonin with the mitochondrial respiratory chain, which is assumed to require only very small, quasi-catalytic amounts of melatonin and would convey antioxidative cell protection as well (Hardeland and Poeggeler, 2005).

Reactive nitrogen species represent another category of potentially destructive substances, which react with melatonin. It was suggested that melatonin acts by binding with calmodulin and suppresses the gene transcription of nitric oxide synthetase enzyme (Leon *et al.,* 2005). The enzyme is involved in a rate limiting step in the synthesis of nitric oxide, a known oxidant suggested to be a major free radical causing follicular damage, thereby resulting in anovulatory condition in summer anoestrus buffalo (Jan *et al.,* 2011). In conclusion, the present study demonstrated the effectiveness of melatonin for inducing estrus and its ability to alleviate oxidative stress in summer anestrus buffalo.

REFERENCES

Bonnefont-Rousselot, D., Collin, F., Jore, D. and Gardès-Albert, M. (2011). Reaction mechanism of melatonin oxidation by reactive oxygen species in vitro. *J. Pineal. Res.,* **50**: 328-335.

Das, G.K. and Khan, F.A. (2010). Summer anoestrus in buffalo. *Reprod. Domest. Anim.,* **45**: 483-494.

Ghuman, S.P.S., Singh, J., Honparkhe, M., Dadarwal, D., Dhaliwal, G.S. and Jain, A.K. (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**: 600-607.

Ghuman S.P.S. (2014). Challenges in Bovine reproduction. In: Ghuman, S.P.S., Gandotra, V.K. and Brar, P.S. (eds.), *Adoption of newer reproductive techniques in education, diagnostics and research*. Advanced Training Course. Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, pp 6-13.

Hardeland, R. and Pandi-Perumal, S.R. (2005). Melatonin, a potent agent in antioxidative defense: actions as a natural food constituent, gastrointestinal factor, drug and prodrug. *Nut. Metabol.,* **2**: 22.

Jan, M.H., Das, G.K., Khan, F.A., Singh, J., Bashir, S.T. and Khan, S. (2014). Evaluation of follicular oxidant-antioxidant balance and oxidative damage during reproductive acyclicity in water buffalo (*Bubalus bubalis*). *Asian Pac. J. Reprod.,* **3**: 35-40.

Karsch, F.J., Bittman EL, Foster DL, Goodman RL, Legan SJ and Robinson JE. (1984). Neuroendocrine basis of seasonal reproduction. *Recent Prog. Horm. Res.,* **40**: 185-222.

Korkmaz, A. and Manchester, L.C. (2011). Reactive nitrogen species: devastating intracellular players and melatonin as a defender. *J. Exp. Integr. Med.,* **1**: 63-65.

Kumar, A., Mehrotra, S., Singh, G., Narayanan, K., Das, G.K., Soni, Y.K., Singh, M., Mahla, A.S., Srivastava, N. and Verma, M.R. (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.

Leon. J., Acuna-Castroviejo, D., Escames, G., Tan, D.X. and Reiter, R.J. (2005). Melatonin mitigates mitochondrial malfunction. *J. Pineal. Res.,* **38**: 1-9.

Misztal, T., Romanowicz, K. and Barcikowski, B. (2002). Melatonin-a modulator of the GnRH/LH axis in sheep. *Reprod. Biol.,* **2**: 267-275.

Nakano, M., Kato, Y. and Tsunoda, Y. (2012). Effect of melatonin treatment on the developmental potential of parthenogenetic and somatic cell nuclear-transferred porcine oocytes in vitro. *Zygote,* **20**: 199-207.

Parmeggiani, A., Di Palo, R., Zicarelli, L., Campanile, G., Esposito, L., Seren, E., Accorsi, P.A. and Soflai, S.M. (1994). Melatonin and reproductive seasonality in the buffalo cow. *Agricol. Ricerca,* **153**: 41-48.

Pieri, C., Moroni, F., Marra, M., Marcheselli, F. and Recchioni, R. (1995). Melatonin is an efficient antioxidant. *Arch. Gerontol. Geriatr.,* **20**: 159-165.

Reiter, J., Tan, D.X., Manchester, L.C. and Qi, W. (2001). Biochemical reactivity of melatonin with reactive oxygen and nitrogen species: a review of the evidence. *Cell Biochem. Biophys.*, **34**: 247-256.

Tamura, H., Takasaki, A., Miwa, I., Taniguchi, K., Maekawa, R., Asada, H. and Sugino, N. (2008). Oxidative stress impairs oocyte quality and melatonin protects oocytes from free radical damage and improves fertilization rate. *J. Pineal. Res.,* **44**: 280-287.

Tomas-Zapio, C. and Coto-Montes, A. (2005). A proposed mechanism to explain the stimulatory effect of melatonin on antioxidative enzymes. *J. Pineal. Res.,* **39**: 99-104.

Zicarelli, L. (1997). Reproductive seasonality in buffalo. *Bubalus Bubalis,* **4**: 29-52.