



Ameliorating postpartum reproductive cyclicity using exogenous melatonin implant in water buffalo (*Bubalus bubalis*)

SAGAR LOCHAN¹, MRIGANK HONPARKHE², RANJNA S CHEEMA³, AJEET KUMAR⁴, S P SGHUMAN⁵ and P S BRAR⁶

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141 004 India

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ABSTRACT

This study was conducted to evaluate the impact of melatonin implants on oxidative stress levels and improving reproductive cyclicity in early postpartum buffalo. Total of 30 buffaloes at 15 days postpartum were randomly divided into melatonin treatment (n=15, one melatonin implant/50 kg body weight, 18 mg melatonin/implant) and control (n=15) groups. Both the groups were equally monitored for overt estrus signs and subjected to trans-rectal ultrasonography to check ovarian status. The blood samples were collected from jugular vein at weekly interval from day 15 to 43 post-partum to assess oxidative stress status. Significant reduction in concentration of malondialdehyde was observed in blood plasma from day 36 postpartum in treatment as compared to control buffaloes. The superoxide dismutase increased in treatment group from day 29 postpartum as compared to control. The concentration of glutathione reductase revealed nonsignificant difference between the groups. Treatment buffaloes showed higher oestrous exhibition rate (66.6% v/s 26.6%) with significant early onset of overt oestrus signs (24.10±1.49 days) compared to control (34.25±5.25 days). Ovulation rate was higher in treatment (n= 13; 86.66%) than that in control (n=8; 53.33%) buffaloes. In conclusion, melatonin implants efficiently reduced oxidative stress and resulted early resumption of ovarian activity, higher oestrus exhibition and ovulation rates in postpartum buffaloes.

Key words: Buffalo, Involution, Melatonin, Oxidative stress, Ovulation

Buffalo is a most valuable livestock resource in Asian countries including India and occupies a critical niche in many agricultural systems, providing milk, meat and work power. Buffalo are the second largest source of milk supply in the world. The reproductive performance of buffalo is restricted as a consequence of various inherent problems like delayed sexual maturity, silent oestrus, reproductive seasonality, poor heat detection, low conception rate, high thermal and lactation stress, postpartum anoestrus and long inter calving interval resulting in economic loss to buffalo breeders (Das and Khan 2010, Kennady *et al.* 2018). Besides these factors, oxidative stress has been identified as one of the important cause of reproductive failure in buffalo as a result of an imbalance between antioxidant system and reactive oxygen species (Megahed *et al.* 2006, Sarwar *et al.* 2009, Khan and Das 2012) leading to prolonged postpartum anoestrus. Melatonin (N-acetyl-5-methoxytryptamine) is the paramount hormone secreted by

the pineal gland only during the dark phase of photoperiod and serves as an antioxidant to protect organisms from pervasive oxidative stresses (Mauriz *et al.* 2013). Melatonin implants appeared beneficial for inducing ovarian cyclicity in buffalo heifers (Ghuman *et al.* 2010), however, such studies are lacking in early postpartum buffalo. We hypothesized that exogenous melatonin during early postpartum would reduce the oxidative stress thereby causing early resumption of reproductive cyclicity in buffaloes. Hence, the objectives of the study were to assess the impact of melatonin implants on oxidative stress, resumption of ovarian activity, oestrus induction and ovulation in recently calved buffaloes.

MATERIALS AND METHODS

Location, animals and management: The study was conducted on 30 normally calved buffalo (mean parity: 2.03±0.14, mean age: 4.85±0.27 years, mean body condition score: 3.083±0.06 and mean body weight: 460±23.26) from the day 15 postpartum. Buffaloes were selected from a private dairy farm, Ludhiana (30.82°N, 75.89°E), Punjab, India during March to August with maximum ambient temperature ranging from 26–39°C and relative humidity from 39–76%. The selected buffaloes were devoid of dystocia, torsion, prolapse and other obstetrical

Present address: ¹MVSc Scholar (sagarlochan000@gmail.com), ²Gynaecologist (honparkhem@rediffmail.com), ³Senior Physiologist (ranjna.cheema@gmail.com), ⁴Associate Professor (ajeetvet@yahoo.com), ⁶Professor (parkashbrar@gmail.com), Department of Veterinary Gynaecology and Obstetrics; ⁵Professor (ghuman_s@yahoo.co.in), Teaching Veterinary Clinical Complex.

emergencies. Prior to start of experiment, buffaloes were subjected to gynaecological examination using ultrasound scanner to rule out pathological conditions of reproductive tract. Normally calved buffaloes, after 15 days postpartum were randomly divided into treatment (n=15) and control (n=15) groups.

Treatment group buffaloes were administered with 2×4 mm size absorbable melatonin implants (18 mg melatonin/implant, Melovine®, CEVA Animal Health Limited, Buckingham, UK) at the base of the ear subcutaneously on day 15 postpartum. Total implants to be inserted were calculated based on body weight of buffalo (One implant/50 kg body weight, Papachristoforou *et al.* 2007). Control group did not receive any treatment.

Ultrasonography (USG) and blood sampling: A battery operated B mode ultrasound scanner with a feature of inbuilt interchangeable 5/7.5 MHz linear array rectal transducer was used for the examination of reproductive tract. Blood samples were collected from jugular vein in heparinized vials from all the buffaloes after completion of each scan and centrifuged at 3000 rpm for 15 min. Plasma was discarded and the remaining haematocrit was washed thrice with Tris 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. Aliquots of haemolysate were prepared and stored at -20°C for further analysis. Both the groups were equally monitored and were subjected to per rectal examination, trans-rectal ultrasonography of uterus and ovaries using linear array B-mode ultrasound equipped with 7.5 MHz for ovarian status. Blood samples were collected on days 15, 22, 29, 36 and 43 post calving.

Estimation of haemoglobin (Hb) and oxidative stress parameters: In the central tube of haemocytometer, 100 µl of 0.1 N HCl was poured followed by addition of 20 µl blood sample. The distilled water was added drop-wise till the colour of the tube matches with the side walls of the haemocytometer and the Hb content in g% was recorded. The prepared RBC lysate was used to analyse oxidative stress parameter, viz. lipid peroxidation (µmole/g Hb; Buege and Steven 1978), superoxide dismutase (unit/g Hb;

Nishikimi *et al.* 1972) and glutathione reductase (IU/g/Hb/min; Krohne-Ehrich *et al.* 1977).

During study period, the buffaloes from both the groups were visually monitored for estrus exhibition twice a day at 12 h interval (morning and evening) and later on confirmation of presence of ovulatory dominant follicle and ovulation (disappearance of ovulatory dominant follicle) was done through transrectal ultrasonography. The size of CL (mm) was measured in ovulated buffaloes (5 days post-ovulation). The interval from calving to first postpartum estrus, estrus exhibition and ovulation rates were also measured.

Statistical analysis: Numerical data are represented as mean±SEM, and differences were considered to be significant at P<0.05. ANOVA with repeated measures was employed between treated and control groups as well as between their ovulated counterparts to determine difference in oxidative stress parameters and the effect of time on each parameter and the treatment. Through One way ANOVA difference in day of exhibition of overt oestrus signs was analysed. Chi-square test was employed for oestrus exhibition rate and ovulation rate. ANOVA with repeated measures was used to determine difference in involution pattern between treatment and control. Statistical analysis was performed using IBM SPSS Advanced Statistics 20.0.

RESULTS AND DISCUSSION

The present study was conducted to assess the effect of melatonin implants on oxidative stress, oestrus and ovulation induction in early postpartum buffaloes. The level of oxidative stress following exogenous melatonin was determined by estimating lipid peroxidation, superoxide dismutase and glutathione reductase activity on different days.

Melatonin, lipid peroxidation and antioxidants enzymes: The mean±SEM Malondialdehyde (MDA; µmole/g Hb) for lipid peroxidation, superoxide dismutase (SOD; U/g Hb) and glutathione reductase (GR; IU/g Hb/min) are given in Table 1.

The lipid peroxidation based on MDA concentration revealed significant (P<0.05) reduction on days 36 and 43

Table 1. Malondialdehyde concentration, Superoxide dismutase and Glutathione reductase in RBC lysate

Group	Day 15	Day 22	Day 29	Day 36	Day 43
<i>Malondialdehyde (MDA, µ mole/g Hb)</i>					
Treatment (n=15)	227.56±26.52	197.93±24.48	172.42±20.10	154.65±19.70 ^{a*}	144.91±17.34 ^{a*}
Control (n=15)	197.45±30.34	217.80±6.04	207.05±17.16	229.67±18.87 ^b	235.89±23.90 ^b
<i>Superoxide dismutase (SOD; U/g Hb)</i>					
Treatment (n=15)	1089.11±344.85	1177.38±241.72	2141.38±332.68 ^{a*}	1964.80±336.30 ^{a*}	1627.40±350.70 ^a
Control (n=15)	746.42±145.66	1263.57±121.18	591.94±157.86 ^{b*}	607.71±128.41 ^{b*}	526.09±106.02 ^b
<i>Glutathione reductase (GR; IU/g Hb/min)</i>					
Treatment (n=15)	4.21±1.76	9.60±3.50	10.20±4.92 [*]	15.58±5.87 [*]	20.63±6.84 [*]
Control (n=15)	6.46±2.47	6.19±2.40	3.80±1.10	5.80±1.50	5.53±1.03

^{a,b}P<0.05. Values with different superscripts within the column differ significantly with each other. *P<0.05, values differ significantly within the row.

in melatonin treatment group as compared to control. The non-significant changes in the levels of MDA in control was observed during different days post-partum (day 15–43) MDA is a strong indicator of lipid membrane damage following oxidative stress. The protective effect of melatonin by diminishing oxidative damage analysed by reduced concentration of oxidative biomarker, MDA has been reported in previous studies (Allegra *et al.* 2003, Reiter *et al.* 2004). Kumar *et al.* (2015) evaluated the effect of sustained-release melatonin on MDA as one of the biomarkers of oxidative stress and observed significant decline in its level following treatment in summer stressed anestrus buffaloes. The reduced level of MDA following melatonin treatment in this study proves its beneficial effects in reducing damage due to oxidative stress in early postpartum buffaloes.

The SOD and GTR activity started increasing from Day 22 postpartum and were significantly higher ($P < 0.05$) in treatment group on day 29 till day 43 as compared to that in control group on similar occasions (Table 1). A significantly declined activity of SOD activity was observed in control buffaloes as the duration postpartum increased while GTR activity in this group remained unchanged.

SOD and GTR are the most important and effective intracellular enzymatic antioxidants (Rahal *et al.* 2014). Melatonin enhances the activity of these antioxidant enzymes and reduces oxidative stress (Ding *et al.* 2014, Hacıyevki and Baba 2018). The increasing trends of SOD and GTR following melatonin treatment in the present study are in accordance with previous study by Singh *et al.* (2016) in summer anoestrus buffaloes.

Melatonin and postpartum reproduction: The impact of exogenous melatonin on postpartum reproduction w.r.t. oestrus exhibition, interval from calving to first postpartum oestrus, and ovulation rate is given in Table 2. The oestrus was exhibited in more ($\chi^2 = 4.821$; $P < 0.05$) number of buffaloes in treatment group (66.6%) as compared to control ones (26.6%). The interval from calving to first postpartum oestrus was shorter ($P < 0.05$) in treatment group than that in control (24 days vs 34 days). The ovulation rate was also higher ($\chi^2 = 3.968$; $P < 0.05$) in treatment group versus control (86.66 vs 53.33). Amongst ovulated buffaloes from treatment group, ten (76.92%) buffaloes exhibited overt oestrous signs but 3 buffaloes failed to exhibit oestrus

whereas, only 4 (50.0%) out of 8 ovulated buffaloes from control group exhibited overt oestrus signs. The size of preovulatory dominant follicle and CL on day 5 post-ovulation were greater ($P < 0.05$) in treatment group (13.36 ± 0.4 mm and 14.38 ± 0.75 mm) as compared to those in control (10.62 ± 0.2 mm and 11.63 ± 1.69 mm), respectively.

The results were in agreement with previous studies conducted on summer anestrus buffaloes using exogenous melatonin implants and recorded high (65–100%) oestrus induction rates (Ghuman *et al.* 2010, Kumar *et al.* 2015, Singh *et al.* 2016). Sustained release melatonin implants maintained the increased circulatory levels of melatonin for day 35–42 post-treatment (Singh *et al.* 2016). Besides controlling seasonal reproduction, melatonin has been proven to be an effective antioxidant and free radical scavenger (Mauriz *et al.* 2013).

Melatonin has potential role in regulating several process, viz. follicular growth and functions, angiogenesis, vasodilation, steroidogenesis and ovulation (Reynolds *et al.* 2002). Melatonin is known to control ovarian activity, attributed to high level of melatonin concentration in follicular fluid and presence of melatonin receptors in granulosa cells (Wang *et al.* 2012, Barros *et al.* 2013) and hence might be responsible for early resumption of postpartum reproduction in buffaloes.

Collectively, these results supported our hypotheses that exogenous melatonin during early postpartum reduced the oxidative stress as indicated by declined MDA levels and high SOD and GTR activity and thereby resulted early resumption of postpartum reproduction in buffaloes.

It is concluded that exogenous melatonin implants are efficient in reducing oxidative stress in buffaloes immediately after parturition and this reduction is associated with early resumption of ovarian activity, higher oestrus induction and ovulation rates. Further studies can be designed in order to reduce voluntary waiting period in buffaloes using melatonin implants prior to suitable fixed time artificial insemination protocol.

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Table 2. Impact of exogenous melatonin on postpartum reproduction w.r.t. oestrus and ovulation induction in postpartum buffaloes

Group	Oestrus exhibition	Interval from calving to first postpartum oestrus (days)	Ovulation rate (%)
Treatment	66.6% (10/15) ^a	24.10 $\pm$ 1.49 ^a	86.66(13/15) ^a
Control	26.6% (4/15) ^b	34.25 $\pm$ 5.25 ^b	53.33(8/15) ^b

^{a,b} $P < 0.05$. Values with different superscripts within the column differ significantly with each other.

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