



Estimation of serum minerals and glucose following subcutaneous melatonin treatment for restoration of ovarian cyclicity in summer anestrus buffaloes (*Bubalus bubalis*)

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ABSTRACT

The present investigation was designed to uncover the effect of melatonin on serum calcium, phosphorus and glucose concentration following restoration of ovarian cyclicity in summer anestrus buffalo. The investigation was conducted on 28 healthy postpartum Murrah buffalo cows of age 5.11 ± 0.62 years having body weight of 539 ± 17.48 kg maintained under isomanagerial conditions with intensive system. Buffaloes diagnosed as summer anestrus (28) on the basis of absence of estrus signs three months postpartum, absence of ovarian activity by rectal examination and serum progesterone assay at 10 days interval. Buffaloes were grouped as untreated (Group I, sterilized corn oil, 8) and treated (Group II, single S/C injection of melatonin (MLT) @18 mg/50 kg b.wt in sterilized corn oil, 20). Estrus detection was carried out twice daily using teaser bull parading along with observation of behavioural estrus signs till day 28 post-treatment. Blood samples were collected at 4-day interval starting from day 8 pretreatment to 28 day post-treatment and for the entire cycle length in responded animals at 4-day interval. Serum calcium, phosphorus and glucose were estimated by commercial kits. Serum Ca, P and glucose concentrations in MLT treated and control buffaloes did not differ significantly within and between both groups. Serum Ca, P and glucose concentrations did not alter significantly between days of MLT induced estrous cycle. However, the group and period effect as well as interaction effect of group and period in serum calcium and phosphorous were not significant whereas, the group and period effect was significant but interaction effect of group and period were not significant in serum glucose concentration. Estrus induction rate was 90% with overall conception rate of 32.4%. Hence, it may be concluded that MLT supplementation restored cyclicity resulting in improvement in conception rate without significantly altering serum calcium, phosphorous and glucose concentration in summer anestrus buffaloes.

Key words: Calcium, Glucose, Melatonin, Ovarian cyclicity, Phosphorous, Summer anestrus

Low reproductive efficiency in the buffalo remains a major economic problem globally due to inherent reproductive problems including delayed sexual maturity, seasonality in breeding, anestrus, long calving interval, silent heat coupled with poor expression of estrus, low conception rate and high thermal and lactation stress (Nanda *et al.* 2003, Terzano *et al.* 2012). Normal reproductive behaviour in domestic animals is closely associated with interaction of hormonal, nutritional as well as minerals status in the body (Newar *et al.* 1999). Minerals play an important role in increasing efficiency of livestock production and reproduction and disturbances in one or

more minerals lead to a cascade of events that alter the hormonal milieu along the hypothalamo-pituitary-ovarian axis which ultimately leads to disturbance in reproductive functions (Kumar *et al.* 2007) and anestrus. Heat stress causes reduction in dry matter intake that leads to negative energy balance and hypoglycemia which leads to anovulation (Roche and Diskin 2001, Hala *et al.* 2009). Plasma glucose is a metabolic signal providing information for control of GnRH release (Foster and Nagatani 1999) and has an important role in ovarian activity.

Melatonin (N-acetyl-5-methoxytryptamine), an indole derivative, synthesized and secreted only during the hours of darkness by pineal gland, is implicated in the sequence of events leading to the onset of puberty and reproductive seasonality in seasonally breeding animals (Malpaux *et al.* 1997). Melatonin implants treatment was successfully exploited for initiating ovarian cyclicity in true anestrus buffalo heifers (Ghuman *et al.* 2010). However, the precise mechanisms involved remain unknown in buffalo. As melatonin interacts with various endocrine systems (Zieba

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et al. 2007), it could be speculated that melatonin treatment may initiate ovarian cyclicity in true anestrus buffalo heifers through its influence on body metabolism (Darul and Kruczynska 2004, Singh *et al.* 2010). Authors have got encouraging finding using subcutaneous melatonin in initiation of ovarian cyclicity with estrus induction rate of 90% and overall conception rate of 32.4% in summer anestrus buffaloes (Kumar *et al.* 2015). Therefore, the present study was envisaged as there is dearth of information pertaining to the effect of MLT on serum minerals and glucose concentration in summer anestrus buffaloes which are key factors involved in ovarian cyclicity.

MATERIALS AND METHODS

Housing and management: Experimental animals were maintained under isomanagerial conditions in a well ventilated brick cemented house with non-slippery floor open byre and offered standard ration that included green and dry fodder along with concentrate and *ad lib.* clean drinking water.

Experimental animals: The proposed study was conducted on 28 healthy postpartum Murrah buffalo cows of 4–7 years age (Mean 5.11 ± 0.62) and body weight 400–690 kg (Mean 539 ± 17.48) maintained at the Cattle and Buffalo Farm, LPM section of the institute during summer of 2013 (April to August). During the study period, THI was 82.34 (Tucker *et al.* 2008) which is not favourable for buffalo reproduction (Upadhyay *et al.* 2009). At first anestrus was diagnosed on the basis of lack of overt signs of estrus for at least 3 months postpartum as detected by teaser bull parading twice a day (late evening and at dawn). Thereafter, serum progesterone assay was done using radioimmunoassay (Immunotech, France) at 10 day interval along with concurrent trans-rectal examination to confirm anestrus condition. Buffalo cows showing lack of overt sign of estrus, smooth ovaries with the absence of corpus luteum and serum progesterone concentration below 1 ng/ml at two consecutive occasions were considered as anestrus.

Experimental design: Melatonin was of analytical grade and procured from Sigma Chemicals, USA. As melatonin implants are costly and not easily available, corn oil (Critser *et al.* 1987) was used as vehicle to dissolve crystalline MLT powder @ 18 mg/ml of corn oil (Coronola containing refined corn oil, Sangrur Agro Ltd., Sangrur, Punjab, India). Once dissolved, the suspension was used on the same day. Summer anestrus buffaloes were grouped as untreated (Group I, control, 8) and treated (Group II, 20). Treated buffaloes were given single s/c injection of MLT @ 18 mg/50 kg body weight (day 0 of treatment) (Ghuman *et al.* 2010). Control animals were administered with corn oil sans MLT. For all the experimental animals, estrus detection was carried out twice daily using teaser bull parading along with observation of behavioural estrus signs till days 28 post-treatment. All the animals showing signs of estrus were artificially inseminated (AI) using frozen semen (20 million spermatozoa/straw) of proven fertility twice at 12 h interval.

Blood sampling and serum parameters estimation: Blood

samples were collected by jugular venipuncture aseptically using 18-G needle at 4-day interval starting from day 8 pretreatment to day 28 post-treatment. Upon detection of estrus (day 0 of estrus cycle), blood was collected for the entire cycle length at 4 day interval, i.e. up to day 24 post-estrus. Serum was separated by centrifugation at 187×9 g for 10 min and stored at -20°C until analysis. Serum calcium, phosphorus and glucose were estimated by Span diagnostic kits (Surat, India).

Statistical analysis: The procedure as described by Littell *et al.* (2006) was followed for the analysis of data. The mean values are expressed as Mean $\pm$ SE. Repeated measures ANOVA was applied using PROC Mixed of SAS 9.2 software (SAS Institute Inc., Cary, NC, USA). Because the data were unequally spaced on different time points, variance-covariance structure SP (POW) and for multiple comparisons, the Tukey test was applied. Associations between different parameters were analysed for statistical significance using Pearson's correlation coefficient using PROC Mixed of SAS 9.2 software. Values with different superscripts indicate significant ($P < 0.05$) difference.

RESULTS AND DISCUSSION

Serum Ca concentrations ranged from 8.55 ± 0.29 to 9.82 ± 0.52 and from 8.00 ± 0.50 to 9.04 ± 0.42 mg% in MLT treated and control buffaloes, respectively and did not differ significantly within and between both groups. Serum Ca concentrations ranged from 8.96 ± 0.22 to 9.89 ± 0.13 mg% without any significant difference between days of MLT induced estrous cycle. Serum P concentrations ranged from 2.86 ± 0.17 to 3.42 ± 0.26 and 2.94 ± 0.22 to 3.24 ± 0.26 mg% in MLT treated and control buffaloes, respectively and did not differ significantly within and between both groups. Serum P concentrations ranged from 4.35 ± 0.2 to 4.93 ± 0.15 mg% without any significant difference between days of MLT induced estrous cycle, although it was non significantly higher on day of estrus.

Serum glucose concentrations ranged from 45.1 ± 2.85 to 54.07 ± 2.76 and 42.67 ± 2.78 to 54.59 ± 2.02 mg% in MLT treated and untreated buffaloes, respectively and did not differ significantly within and between both groups. Serum glucose concentrations ranged from 54.19 ± 2.72 to 61.50 ± 2.01 mg% without a significant difference in MLT induced estrus cycle which was nonsignificantly higher on day of the induced estrus. However, the group and period effect as well as interaction effect of group and period in serum calcium and phosphorous were not significant. Whereas, the group and period effect was significant but interaction effect of group and period were not significant in serum glucose concentration.

Singh *et al.* (2010) reported no alteration in serum Ca concentration following MLT treatment in delayed pubertal summer anestrus buffalo heifers, which was in agreement with present finding, though initiation of ovarian cyclicity has significant positive correlation with plasma Ca and P concentration (Shah *et al.* 2003). In present study, serum Ca concentration was nonsignificantly higher on day of

estrus, indicating its important role in initiation of ovarian cyclicity (Shah *et al.* 2003). MLT being more potent antioxidant than vitamin E/Se, had no significant effect on serum Ca concentration, which was in agreement with findings of Hala *et al.* (2009) who reported that antioxidants (Zn, Vitamin E/Se) have no significant effect on serum Ca levels in heat stressed anestrus buffaloes.

No significant variation in Serum Ca concentration during different phases of estrous cycle in buffalo had been reported by Sharma *et al.* (1999), which was in agreement to the present investigation. Earlier studies have found nonsignificant higher serum Ca level in cyclic animals compared to anestrus buffaloes (Jani *et al.* 1995) which indicates role of Ca in changes associated initiation of ovarian cyclicity. Serum Ca levels had been reported to be lower in anestrus buffaloes as compared to normal cyclic animals (Singh *et al.* 2006, Chaurasia *et al.* 2010). Serum Ca concentrations vary significantly with normal ovulation, anovulation and delayed ovulation in bovines (Das *et al.* 2009) suggesting a possible role of the ion in the reproductive physiological process.

At hypothalamic level, Ca regulates the neuronal circuitry for GnRH pulsatile secretion (Van *et al.* 2000) and involved in GnRH stimulated LH and FSH secretion from pituitary (Simpson *et al.* 1989), have a role in ovarian steroidogenesis (Veldhuis and Klase 1982) and the disruption of cumulus cell cohesiveness by regulating the number of gap junctions between the cells (Peracchia 1978). An optimum Ca concentration is required for sensitization of reproductive organs through various hormones and the maintenance of normal reproductive cycle.

There was no significant difference in serum P concentration following MLT treatment in postpartum summer anestrus buffaloes in present study, which was in agreement with finding of Singh *et al.* (2010) who observed that MLT implants have no significant effect on plasma P concentration in delayed pubertal summer anestrus buffalo heifers. MLT as being more potent antioxidant than vitamin E/Se, had no effect on serum P concentration in current investigation. However, Hala *et al.* (2009) reported that antioxidants (Zn, vitamin E/Se) treatment lead to significantly increased serum P concentration in heat stressed anestrus buffaloes. However, serum P concentration was higher on day of estrus in present study, indicating its important role in initiation of ovarian cyclicity (Shah *et al.* 2003).

Several researchers have recorded higher serum P values in normal cyclic compared to anestrus cattle and buffaloes (Sharma *et al.* 1999, Dutta *et al.* 2001, Chaurasia *et al.* 2010). Classical manifestations of P deficiency on reproductive processes involve alterations in estrus and decreased ovarian activity (Ahmed 2007) characterized by anestrus, delayed maturity, subestrus and irregular cycles. Low P level leads to imbalance of Ca and P (McClure 1965), causing lower fertility or infertility as these ions play a significant role in the process of reproduction (Little 1970). P is associated with normal function of all animal tissues

by virtue of its role in the process of energy exchange and deficiency causes disturbance in the pituitary-ovarian axis leading to aberrations in the normal reproductive rhythm and leads to anestrus (Sharma *et al.* 1999).

Serum glucose concentration was nonsignificantly different between groups, which was in accordance with the finding of Singh *et al.* (2010) who observed no alteration in plasma glucose concentration in delayed pubertal summer anestrus buffalo heifers following MLT implants. Serum glucose was higher on day of estrus compared to luteal phase and anestrus animals which was in accordance with the findings of Umesh *et al.* (1995), Kumar *et al.* (2010).

Jani *et al.* (1995) and Singh *et al.* (2006) also reported and agreed that negative energy status is known to negatively affect the pituitary functions. Similarly, Dhoble and Gupta (1979) observed and inferred that low level of blood glucose may be an indication of subnormal energy status leading to negative energy balance, therefore, can be used to assess the reproductive functions in buffaloes. In present study, nonsignificantly higher concentration of glucose on day of estrus may suggest that serum glucose may have an important metabolic factor responsible for initiation of ovarian cyclicity following MLT treatment. McClure (1965) observed that variations in blood glucose were clearly linked to cyclicity and fertility as hypoglycemic condition has been found to depress the hypothalamic function leading to loss of ovarian activity due to failure of release of gonadotrophic release (Parkinson 2009).

In conclusion, results of study suggest that blood serum minerals and glucose were not significantly altered following s/c MLT treatment in summer anestrus buffalo, though treatment was able to induce ovarian cyclicity in 90% of the buffaloes with overall conception rate of 32.4%. Therefore, it could be concluded that cyclicity and acyclicity in buffaloes is the resultant of interplay of hormones which in turn seems to be governed by the nutritional status of the animals. Further studies investigating underlying reproductive endocrine mechanisms involved at molecular level in MLT-induced onset of ovarian cyclicity in summer anestrus buffalo are needed.

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