SHORT COMMUNICATION

# Subcutaneous Administration of Melatonin Resumes the Cyclicity and Improves Conception Rate in Anestrus Buffalo Heifers

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## Abstract

The present study was aimed to see the effect of subcutaneous injection of melatonin @ 18 mg/50 kg b.wt. on estrus induction and conception rate in anestrus buffalo heifers (n=24). Among the heifers treated with melatonin (n=14), 6 (42.86 %) animals showed behavioral estrus within 4-28 days of treatment as compared to none in control group (n=10). The first service conception rate was 33.33 % (2/6) in the melatonin treated buffaloes, while none of the buffalo exhibited estrus/conceived in control group. It is concluded that melatonin @ 18 mg/50 kg b.wt. can be used to induce the estrus in anestrus buffalo heifers with satisfactory conception rate.

**Key words:** Anestrus, Buffalo, Conception, Estrus, Melatonin.

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## Introduction

Buffalo has a high productive potential but blamed for poor reproductive performance reflected by various inherent reproductive problems like delayed sexual maturity, silent estrus, reproductive seasonality, problems of heat detection, low conception rate, high thermal and lactation stress, anestrus, and longer inter-calving intervals, as a consequence the productivity is adversely affected (Nanda *et al.,* 2003; Das and Khan, 2010). Buffalo shows behavioural estrus throughout the year, but distinct pattern of breeding season characterized by poor estrus expression during summer season has been reported (Singh *et al.,* 2000; Perera, 2011). The seasonality is important factor for poor reproductive efficiency in buffalo. Reproductive seasonality is governed by the melatonin, a pineal gland hormone (Malpaux *et al.,* 2001). The rise in circulating melatonin, through its action both on hypothalamus and pituitary, is responsible for the increase in plasma concentrations of GnRH and gonadotropins, thus leading to follicular growth and ovulation (Ghuman *et al.,* 2010). The melatonin has an important role in resumption of cyclicity in buffalo (Kumar *et al.,* 2016; Kavita *et al.,* 2018). Melatonin was used to initiate ovarian cyclicity in true anestrus buffaloes (Ghuman *et al.,* 2010; Kumar *et al.,* 2016). There are meagre reports on the effect of melatonin as a sustained release injection on the resumption of cyclicity and conception rate in buffalo heifers. Therefore, the present investigation was designed to examine the effect of slow release (subcutaneous injection) melatonin on ovarian cyclicity and conception rate in anestrus buffalo heifers.

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## Material and Methods

The study was conducted on 24 Mehsana buffalo heifers, 3-4 years of age and 300-435 kg b.wt., maintained at Livestock Research Station, Sardarkrushinagar Dantiwada Agricultural University (SDAU), Dantiwada, Gujarat (India) during 2018 - 2019. All the buffalo heifers (n=24) were subjected to gynecological examination (trans-rectal palpation and ultrasound) before inclusion in the study. Buffalo heifers showing lack of overt signs of estrus and absence of corpus luteum were considered as delayed pubertal anestrus. The anestrus buffalo heifers were divided into two groups;

**Table 1:** Mean (±SE) serum concentration of progesterone ($P_4$) and melatonin in anestrus buffalo heifers.

| Group | Progesterone ($P_4$, ng/mL) | | Melatonin (MLT, pg/mL) | |
|---|---|---|---|---|
| | On the day of treatment | On day 28th / estrus | On the day of treatment | On day 28th / estrus |
| **Treatment (n=14)** | 0.36±0.08 | 0.42±0.12 | $16.28±1.08_a$ | $121.14±1.52^A_b$ |
| **Control (n=10)** | 0.48±0.14 | 0.52±0.15 | 17.32±1.41 | $18.13±0.96^B$ |

Means with superscript (A, B) differ significantly ($p<0.05$) within the column, and those with subscript (a, b) differ significantly ($p<0.05$) within the row.

Group I (n=14) was treated with melatonin (MLT, Himedia Laboratory Pvt. Ltd., Cat#RM1831) s/c injection @ 18 mg/50 kg b. wt. dissolved in corn oil (Kumar *et al*., 2016), while Group II (n=10, control) was treated with corn oil without melatonin. Crystalline melatonin powder at 18 mg/mL of corn oil was dissolved at room temperature. Once dissolved, the suspension was used on the same day.

The estrus detection was done twice daily using teaser bull parading along with observation of behavioural estrus signs till day 28 post-treatment. All the animals showing signs of estrus were artificially inseminated (AI) using frozen semen of proven fertility. Blood was collected from jugular vein in a plain vacutainer before and after treatment and serum was separated by centrifuging at 1000 x *g* for 5 min. The serum progesterone (Calbiotech Inc., Esdoomiaan, Netherland) and melatonin (MT ELISA Kit, Fine Test, Wuhan Fine Test Biotech Co. Ltd.) were assayed on the day of treatment and day of estrus or day 28 post-treatment, whichever was earlier, by using commercially available ELISA kits. Pregnancy diagnosis was done by trans-rectal examination at two months after insemination. Estrus induction rate, interval, and first service conception rate were calculated.

The data were checked for homoscedescity using Shapiro-Wilk test. The two-way ANOVA was used to see the difference of progesterone and melatonin concentration before and after treatment as well as between the groups. Data were expressed as mean ±SE. Level of significance was set at 95%. Statistical analysis was done using SPSS software (IBM® SPSS® statistics, version 20.0).

## Results and Discussion

Six of fourteen (42.86%) melatonin treated anestrus Mehsana buffalo heifers exhibited behavioral estrus with 4-28 days of estrus induction interval. Ghuman *et al.* (2010) found 100 % ovulatory estrus induction response using MLT implants in delayed pubertal summer anestrus buffalo heifers. Kumar *et al.* (2016) reported 90.0% estrus induction rate with average interval of 18.06±1.57 days and 32.4 % conception rate following slow-release melatonin supplementation in postpartum anestrus buffaloes.The low estrus induction rate in the present study compared to other workers may be due to variation in estrus detection aids used in the different studies. The cascading effect of increased MLT on hypothalamus and pituitary explains the resumption of cyclicity in anestrus buffaloes (Yang *et al.,* 2021). Melatonin increases the number of large follicles and diameter in summer anestrus buffalo heifer (Ramadan *et al.,* 2014). The variation in the onset of estrus may be due to the presence or absence of dominant follicle at the time of melatonin administration and individual variation of attaining the threshold level to activate the hypothalamo-pituitary-gonadal axis for induction of estrus (Ghuman *et al.,* 2010). The first service conception rate was 33.33 % (2/6) in MLT treated buffalo heifers, while none of buffaloes exhibited the behavioral signs of estrus or conception in control group during the study period. The present conception rate in melatonin treated anestrus buffaloes heifers corroborated with the report of Kumar *et al.* (2016) in melatonin treated postpartum anestrus buffaloes. The high percentage of conception rate has also been reported in the melatonin-implanted anestrus sheep by Forcada *et al.* (2006).

The $P_4$ concentration did not differ significantly between the groups (Table 1). The MLT concentration was significantly ($p<0.05$) increased after the melatonin treatment in group I. Kumar and Purohit (2009) reported significantly higher estrus induction and pregnancy rate in seasonal anestrus goats treated with MLT. The increased MLT concentration in the treatment group than the control explains the induction of estrus might be through action at hypothalamo-pituitary-ovarian axis (Ramadan *et al.,* 2014).

In conclusion, s/c injection of melatonin @ 18 mg/50kg b.wt. induced behavioral signs of estrus in anestrus buffalo heifers with satisfactory conception rate.

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