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Effectiveness of melatonin and controlled internal drug release device treatment on reproductive performance of buffalo heifers during out-of-breeding season under tropical conditions

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ABSTRACT

Sixteen Murrah buffalo heifers, divided into control and treatment groups of eight animals each, were used to study the effect of melatonin and controlled internal drug release (CIDR) device treatment on the resumption of ovarian activity during out-of-breeding season (summer solstice). Treated group was implanted with melatonin (18 mg of melatonin per 50 kg of body weight) for 45 days and then heifers of both groups received CIDR for 9 days. All heifers received intramuscular 500 IU eCG on the day before CIDR removal and 10 μg GnRH on the day after CIDR withdrawal. All animals were subjected to estrus detection daily. Blood sampling in conjunction with transrectal ultrasonography were performed twice weekly to determine serum concentrations of melatonin, progesterone, LH, and antioxidant enzyme activities, as well as to monitor the ovarian follicular activity. Melatonin treatment resulted in an increase ($P < 0.01$) in serum melatonin and a decrease ($P < 0.01$) in serum progesterone and LH. In addition, melatonin had no significant effect on the frequency of LH pulses. Furthermore, melatonin treatment increased ($P < 0.01$) the diameter of the largest follicle and the number of large follicles between Days 0 and 35 of melatonin treatment. However, melatonin exhibited superior ability to maintain CL at 21 days after artificial insemenation (AI) and increased the percentage of conception to threefold higher than control. In conclusion, melatonin implantation successfully improved the diameter of largest follicles and the ability to maintain CL at 21 days after AI in buffalo heifers during out-of-breeding season under tropical conditions.

1. Introduction

Although buffaloes are polyestrous, their ovarian cyclicity and conception rates show wide variations throughout the year [1–3]. This weak seasonality does not seem to depend on diet and fodder availability or metabolic status, but climate, in particular photoperiodism, plays a pivotal role [4]. The proportion of buffaloes exhibiting estrus during short day length period is considerably greater than that during the period of long day length, indicating that decreased daylight is a stronger determinant of the resumption of ovarian activity [2]. The Mediterranean buffaloes showing seasonal reproductive trends have highest night-time plasma melatonin concentrations in winter and lowest in summer [4,5]. Melatonin is

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implicated in the sequence of events leading to the onset of puberty in cow heifers [6].

Melatonin (N-acetyl-5-methoxytryptamine) is synthesized by the pineal gland during the dark phase of photoperiod [7]. Melatonin conveys photoperiodic information to synchronize cell physiology with the dark-light cycle [7]. Moreover, melatonin stimulates gene expression of antioxidative enzymes such as superoxide dismutase, glutathione peroxidase, catalase, and glutathione reductase [8]. Recently, melatonin implant treatments were successfully exploited for initiating ovarian cyclicity in anestrus buffalo heifers [9,10]. However, the precise mechanisms involved remain unknown.

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 [11,12]. However, estradiol seems to be positively linked with the action of melatonin on reproductive activity in which melatonin exerts a modulatory effect on LH secretion, stimulating the release of this gonadotropin in the presence of estradiol feedback (cyclic animals) and inhibiting it during steroid deprivation (anestrus animals) [13].

In addition to melatonin, the application of estrus/ovulation protocols suggests that controlled internal drug release (CIDR) was better for anestrus buffaloes. The priming of hypothalamohypophysial pituitary gonadal (HPG) axis with adequate amounts of progesterone is beneficial for the recovery of HPG axis function, and hence, a better display of estrous behavior by anestrus buffaloes at induced estrus [14,15]. Furthermore, the sufficient priming of endometrium with progesterone may be necessary to enhance the conception rate [16]. In addition, the effect of the pineal hormone on embryo quality could be produced at the oocyte stage, because melatonin improves developmental oocyte competence in the seasonal anestrus period [17] and increases significantly the maturation rate of oocytes and tends to increase their cleavage rate [18].

The use of melatonin followed by CIDR in inducing ovarian activity in buffaloes has not been done previously. Therefore, the objective of this study was to assess the efficacy of melatonin implants followed by CIDR treatment for alleviation of summer-induced decline in ovarian activity in anestrus buffalo heifers and to enhance the maintenance of CL to improve the conception rate.

2. Materials and methods

The present study was conducted at the animal farm (29° 10′ N, 75° 41 E), Central Institute for Research on Buffaloes, Hisar, India, using anestrus buffalo heifers during the out-of-breeding season (from of April to July). All procedures and experimental protocols were conducted in accordance with the "Guide for the Care and Use of Agricultural Animals in Research and Teaching," Federation of Animal Science Societies [19].

2.1. Animals and management

Sixteen Murrah buffalo heifers (age 36.06 ± 0.69 months and mean weight 348.81 ± 8.54 kg) were used in the present study. The study was conducted during the hot-humid months from last week of April to July when ambient temperatures and relative humidity ranged from 33 °C to 45 °C and 35% to 80%, respectively. Heifers were confined for the entire period of study to a barn with access to an open sheltered space. All heifers included in this study had attained the pubertal age. They were subjected to teasing twice daily for estrus detection, but failed to exhibit estrus. They were fed on roughage and concentrate supplement according to their body weight requirements [20]. Chaffed green fodder and wheat straw were offered in summer. Water was offered in excess to animals at all times. Heifers were free from diseases and were clinically normal with a healthy appearance. They were subjected to gynecologic examination before inclusion in the study, and those diagnosed with any pathologic condition of the reproductive tract were excluded after ultrasonography.

2.2. Experimental design

Heifers were randomly allocated to melatonin non-implanted (control) and implanted (treated) groups (n = 8 each). In melatonin-treated group, heifers were administered 2 × 4 mm absorbable melatonin implants (18 mg melatonin per implant, Regulin; CEVA Animal Health Limited, Chesham, Buckinghamshire, UK) at the base of left ear using an implanter. Total implants inserted to each heifer were calculated on the basis of their body weight (one implant per 50 kg, [21]). These implants were designed to release melatonin for at least 60 days, although their functionality can extend to more than 100 days without disturbing the endogenous secretion of melatonin as seen in ewes [22,23]. On Day 45 after melatonin implantation, all implanted heifers were treated with Eazi-Breed CIDR (1.38 g of progesterone; Pfizer Animal Health, Auckland, New Zealand) for 9 days (removed on Day 54) and were intramuscularly treated with 500 IU eCG (Folligon; Intervet International, Boxmeer, Netherlands) on the day before CIDR removal (Day 53). All animals were examined daily for estrus detection and were intramuscularly treated with 10 μg GnRH (Receptal; Intervet International) at the time of insemination (Day 56).

2.3. Ultrasonography and blood sampling

Ovarian ultrasonography was carried out with a B-mode ultrasound scanner (Toshiba, SSA 220, Just Vision; Medical Systems Corporation, Tochigi-ke, Japan) equipped with 5 to 7.5 MHz linear-array rectal transducer (ALR 575 probe; ECM). Blood samples were collected *via* jugular venipuncture in a heparinized vial after each scan. Plasma was separated immediately and kept in two aliquots at −20 °C until analysis. Ultrasonography and blood sampling were conducted twice a week throughout the period of melatonin treatment, then on the day of CIDR insertion (Day 45) and removal (Day 54) and each day of hormonal injection (Day 53 for eCG and Day 56 for GnRH, respectively). After insemination (which was carried out on Days 55 and 56), ultrasonography and blood sampling were achieved on Days 65, 75, and 84 (10, 21, and 30 days after AI) to follow up formation and maintenance of CL pregnancy diagnosis.