Theriogenology 86 (2016) 1048–1053

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Manipulation of reproductive performance of lactating buffaloes using melatonin and controlled internal drug release device treatment during out-of-breeding season under tropical conditions

T.A. Ramadan [a,*], R.K. Sharma [b], S.K. Phulia [b], A.K. Balhara [b], S.S. Ghuman [c], I. Singh [b]

[a] Agricultural Research Center, Animal Production Research Institute, Giza, Egypt
[b] Division of Animal Physiology and Reproduction, Central Institute for Research on Buffaloes, Hisar, Haryana, India
[c] Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

ARTICLE INFO

Article history:
Received 8 October 2015
Received in revised form 20 March 2016
Accepted 21 March 2016

Keywords:
Melatonin
CIDR
Lactating buffalo
Ovarian activity
Antioxidant enzyme activity

ABSTRACT

Twelve lactating Murrah buffalo, divided into control and treatment group of six 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 implanted with melatonin (18-mg melatonin/50-kg body weight) for 45 days and then animals of both groups received CIDR for 9 days. All animals received intramuscular 500 IU eCG, at day before CIDR removal, and 10-μg GnRH at day after CIDR withdrawal. All animals were subjected to estrus detection daily. Blood samples in conjunction with transrectal ultrasonography were performed once a week to determine serum concentrations of melatonin, progesterone, and antioxidant enzyme activities, as well as to monitor the ovarian activity. Melatonin treatment resulted in an increase ($P < 0.01$) in the overall mean superoxide dismutase activity. Melatonin and CIDR increased the diameter of CL ($P < 0.01$) and plasma progesterone concentration ($P < 0.05$). In addition, melatonin and CIDR exhibited superior ability to maintain presence of CL at Day 21 and Day 30 after artificial insemination and achieved higher percentage of conception rate than control. In conclusion, the CIDR treatment preceded by melatonin improved the reproductive performance in lactating buffaloes during out-of-breeding season under tropical conditions.

1. Introduction

Seasonality in buffalo reproduction has been reported from India, Pakistan, and many other parts of the world [1] which has been attributed to environmental factors more directly than the genetic factors [2]. Buffaloes show seasonality patterns in the breeding behavior. However, in cooler months, that is, from October to March, reproduction is enhanced in buffalo especially in tropical climates, and this period is referred to as the breeding period. Fertility is compromised if out of season occurs. Embryonic mortality has been estimated to be 20% to 40% and is considered to be one of the major causes of fertility loss [3]. Size of CL has also been reported to be smaller during the summer season [4] as compared with the winter season which may contribute to the lesser progesterone production and greater rate of embryonic mortality.

Melatonin is an indole derivative endogenous compound secreted rhythmically by the pineal gland in the brain and plays a major role in regulating the circadian clock and

* Corresponding author. Tel.: +201114022381; fax: +2035916223.
E-mail address: tamereweda1174@Yahoo.com (T.A. Ramadan).

0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.03.034

seasonal reproduction in mammals [5]. More recent studies have reported that, besides its multiple actions on different physiological processes, melatonin as well as its metabolites are indirect antioxidants and powerful direct scavengers of free radicals [6]. The antioxidants protect luteal cells against oxygen radicals produced during steroidogenesis [7]. These oxygen radicals may also be functional in leading to luteolysis and apoptosis in CL during each reproductive cycle [8]. It has been reported that the steroidogenic capacity of the bovine CL shows major changes during the estrous cycle [9], but whether the same protective mechanisms against oxidative damage prevail at different phases of CL development in the buffalo have not been determined. The relatively smaller CL in buffalo may be responsible for the lesser progesterone secretion which may contribute to the greater rate of embryonic mortality [10]. Increases in CL diameter from Day 5 to 10 and progesterone concentration from Day 10 to 25 after artificial insemination (AI) has been reported to be greater in pregnant than in nonpregnant buffalo indicating the importance of attainment of adequate CL size for maintenance of pregnancy [11]. Lesser plasma progesterone concentrations from Day 10 to 20 after AI in buffalo was reported to be associated with greater embryonic mortality [12] indicating the role of CL for establishment of pregnancy.

In addition to melatonin, the application of estrus/ovulation protocols suggests that controlled internal drug release (CIDR) was better for anestrus buffalo hiefiers [13]. The priming of hypothalamo-hypophysial 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]. Also, in buffaloes, treatment with GnRH (gonadoreline) has been shown to induce ovulation within 48 hours, subsequent with an increase in plasma progesterone concentration within 72 and 96 hours after GnRH injection [17]. 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 lactating buffaloes and to enhance maintenance of CL to improve 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 lactating buffaloes during the out-of-breeding season (from June to September). Characteristics of estrus were recorded daily. Buffaloes were observed for visual signs of estrus, twice per day for 1 hour each. 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 [18].

2.1. Animals and management

Twelve lactating Murrah buffalo (parity: 2–4, body condition score: 4–5, and body weight: 400–500 kg) at Day 65 to 70 of lactation and milk yield of 7 to 9 kg/day were used in the present study. The study was conducted during the hot-humid months from first week of June to September when ambient temperatures and relative humidity ranged from 35 °C to 45 °C and 35% to 80%, respectively. Lactating buffaloes were confined for the entire period of study to a barn with access to an open-sheltered space. 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 [19]. Chaffed green fodder and wheat straw were offered in summer. Water was offered in excess to animals at all times. Animals were free from diseases and were clinically normal with a healthy appearance. Lactating buffaloes were milked twice a day, morning (04:00 a.m.) and evening (03:00 p.m.). They were subjected to gynecological examination before inclusion in the study, and those diagnosed with any pathological condition of the reproductive tract were excluded after ultrasonography. Before starting the trial, the ovarian activity was assed ultrasonographically and on the basis of the absence of a functional CL, the animals were categorized as noncycling.

2.2. Experimental design

Lactating buffaloes were randomly allocated to melatonin nonimplanted (control) and implanted (treated) groups (n = 6 each). In melatonin-treated group, animals were administered 2 × 4-mm absorbable melatonin implants (18-mg melatonin/implant, Regulin, CEVA Animal Health Limited, Chesham, Buckinghamshire, UK) at the base of left ear using an implanter. Total implants inserted to each animal were calculated on the basis of their body weight (one implant/50 kg, [20]).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 [21,22]. On Day 45 after melatonin implantation, all animals were treated with Eazi-Breed CIDR (1.38 g of progesterone; Pfizer Animal Health, New Zealand) for 9 days (removed on Day 54) and were intramuscularly treated with 500 IU eCG (Folligon; Intervet, International, Boxmeert, Netherlands) on the day before CIDR removal (Day 53). All animals were subjected daily for estrus detection and were intramuscularly treated with 10-μg GnRH (Receptal; Intervet, International, Boxmeert, Netherlands) of the second insemination in which insemination has been carried out on Days 55 and 56. Experimental layout is presented in Figure 1.

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 (at 06:00 a.m.). Plasma was separated immediately by centrifugation and kept in two aliquots at −20 °C until analysis. Before centrifugation, an aliquot of whole blood