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Equine chorionic gonadotropin improves the efficacy of a timed artificial insemination protocol in buffalo during the nonbreeding season

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ABSTRACT

Two experiments were conducted to evaluate the effects of equine chorionic gonadotropin (eCG) treatment on ovarian follicular response, luteal function, and pregnancy in buffaloes subjected to a timed artificial insemination (TAI) protocol during the nonbreeding season. In experiment 1, 59 buffalo cows were randomly assigned to two groups (with and without eCG). On the first day of the synchronization protocol (Day 0), cows received an intravaginal progesterone (P4) device plus 2.0 mg estradiol benzoate im. On Day 9, the P4 device was removed, all cows were given 0.150 mg $PGF_{2\alpha}$ im, and half were given 400 IU eCG im. On Day 11, all cows were given 10 μg of buserelin acetate im (GnRH). Transrectal ultrasonography of the ovaries was performed on Days 0 and 9 to determine the presence and diameter of the largest follicle; between Days 11 and 14 (12 hours apart), to evaluate the dominant follicle diameter and the interval from device removal to ovulation; and on Days 16, 20, and 24 to measure CL diameter. Blood samples were collected on Days 16, 20, and 24 to measure serum P4. In experiment 2, 256 buffaloes were assigned to the same treatments described in experiment 1, and TAI was performed 16 hours after GnRH treatment. Pregnancy diagnosis was performed by ultrasonography 30 days after TAI. Treatment with eCG increased the maximum diameter of dominant follicles ($P = 0.09$), ovulation rate ($P = 0.05$), CL diameter ($P = 0.03$), and P4 concentrations ($P = 0.01$) 4 days after TAI, and pregnancy per AI (52.7%, 68/129 vs. 39.4%, 50/127; $P = 0.03$). Therefore, eCG improved ovarian follicular response, luteal function during the subsequent diestrus, and fertility for buffalo subjected to a TAI synchronization protocol during the nonbreeding season.

1. Introduction

Buffalo exhibit seasonality in breeding activity and become sexually active in response to a decreasing day length in late summer to early autumn [1]. As latitude decreases, calving is more concentrated during the breeding season, providing rapid re-establishment of postpartum ovarian activity and conception [2]. Therefore, during the nonbreeding season, buffalo often exhibit a high anestrous incidence, which extends the calving to conception interval and, consequently, reduces reproductive performance [3]. Consequently, hormonal treatments have been designed to control both luteal and follicular functions, providing exciting possibilities for synchronization of follicular growth and ovulation to enable the use of timed artificial insemination (TAI) during the nonbreeding season [4–8].

Satisfactory pregnancy rates (approximately 40% to 60%) [7,9–12] have been achieved with the Ovsynch protocol

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(Day 0, GnRH; Day 7, PGF2α; Day 9, GnRH; TAI 16 hours after the second GnRH injection) [13] in cycling buffalo synchronized during the breeding season. However, anestrous buffalo respond poorly to the Ovsynch protocol [8,12,14,15] and have lower pregnancy rates after TAI during the nonbreeding season [16].

In previous studies in postpartum anestrous cows, exogenous progesterone (P4) increased LH pulse frequency during and after the treatment period [17]. In addition, in comparison with untreated animals, this increased follicular fluid and circulating estradiol concentrations, LH pulsatile release, and numbers of LH receptors of granulosa and theca cells in preovulatory follicles [18].

Treatment with intravaginal P4 devices combined with eCG at device removal has been extensively used in *Bos indicus* cattle (reviewed in [19]) and increased ovulation rates, plasma P4 concentrations, and pregnancy rates for suckled beef cows with a high prevalence of anestrous or a low body condition score (BCS) [19–24]. Similar to bovine cows, eCG treatment has been suggested as an effective tool for increasing pregnancy outcomes using TAI protocols in buffalo herds with a high incidence of postpartum anestrous [4,14,25,26]. However, there are few reports of ovarian follicle response and luteal function in the subsequent diestrus of lactating buffalo cows treated with eCG and subjected to a TAI protocol during the nonbreeding season.

Thus, the aim of the present study was to evaluate the effects of eCG on ovarian response, luteal function, and pregnancy outcomes in buffalo (*Bubalus bubalis)* subjected to P4 plus estradiol benzoate treatment for ovulation synchronization using TAI protocol. Our hypothesis was that the eCG treatment would effectively increase ovulation rates, CL diameter, and circulating P4 concentrations in the subsequent diestrus and pregnancy per TAI during the nonbreeding season in buffalo.

2. Materials and methods

2.1. Experiment 1

2.1.1. Animals and management

Experiment 1 was conducted at the Santa Helena farm (Sete Barras, SP, Brazil) during the nonbreeding season (spring through summer; November 2009 to March 2010). In this period, the minimum and maximum temperature were 22.0 °C and 31.9 °C and rainfall was 28.2 mm. Fifty-nine lactating buffalo (*Bubalus bubalis*) were assigned into one of two treatment groups (with eCG, N = 29, and without eCG, N = 30) according to parity (2.2 ± 0.4), days after parturition (170.0 ± 15.4), ovarian activity (ultrasonographic examinations performed to confirm the absence of a CL on Day 0) and BCS (3.0 ± 0.1; scale of 1 to 5, where 1 = very thin and 5 = very fat). Cows had contact with their calves only during milking. These buffaloes were maintained on a *Brachiaria decumbens* pasture with free access to water and mineralized salt.

2.1.2. Experimental design

All buffaloes received an intravaginal P4 device (1.0 g P4; DIB; MSD Animal Health, Sao Paulo, SP, Brazil) plus 2.0 mg of estradiol benzoate (EB; im; Gonadiol; MSD Animal Health) at random stages of the estrus cycle (designated Day 0). On Day 9, the DIB was removed, a luteolytic dose of $PGF_{2\alpha}$ im (0.150 mg days-cloprostenol; Preloban; MSD Animal Health) was given, and the buffaloes were allocated into two groups. One group received 400 IU of equine chorionic gonadotropin (eCG Folligon; im; MSD Animal Health) whereas the other group (without eCG) did not receive any additional treatment. After 2 days (Day 11, approximately 48 hours after device removal), buffalo of both groups received 10 μg of buserelin acetate (GnRH; im; Conceptal; MSD Animal Health; Fig. 1). Blood samples were collected from all cows to measure (RIA) serum P4 concentrations on Days 16, 20, and 24.

2.1.3. Ultrasonographic examinations

Transrectal ultrasonography of the ovaries was performed using a 7.5 MHz linear-array transducer (Mindray DP-2200Vet; Shenzhen, China) on Days 0 and 9 to assess the diameter of the largest follicle, between Days 11 and 14 (12-hour intervals) to evaluate dominant follicle diameter and the interval from the device removal to ovulation, and on Days 16, 20, and 24 to measure CL diameter. The growth rate of the dominant follicle (mm/d) was defined as the difference between the dominant follicle recorded on Day 11 and on Day 9, divided by two. Ovulation was considered to have occurred when a large follicle, previously observed, was no longer present at the subsequent ultrasonographic examination.

2.1.4. Blood sampling and P4 assays

Blood samples were collected by jugular venipuncture after the ovulation synchronization by GnRH administration (Days 16, 20, and 24 from the start of treatment). The samples were refrigerated (4 °C) for 4 to 5 hours after

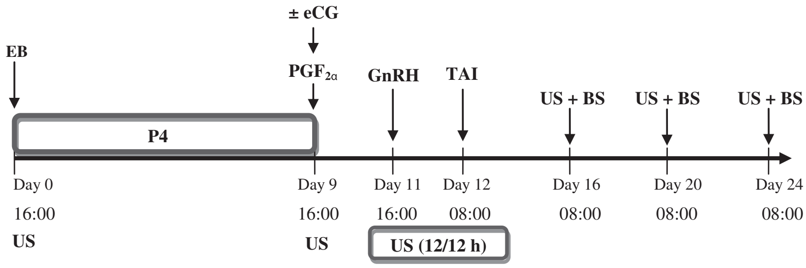

Fig. 1. Schematic diagram of the treatments with or without eCG to synchronize ovulation in buffaloes. BS, blood sample; EB, 2.0 mg estradiol benzoate; eCG, 400 IU equine chorionic gonadotropin; GnRH, 10 μg buserelin acetate; $PGF_{2\alpha}$, 0.150 mg days-cloprostenol; US, ultrasonographic examination.