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Dynamics of follicular growth and progesterone concentrations in cyclic and anestrous suckling Nelore cows (*Bos indicus*) treated with progesterone, equine chorionic gonadotropin, or temporary calf removal

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ABSTRACT

The objective of this study was to investigate the effects of eCG and temporary calf removal (TCR) associated with progesterone (P4) treatment on the dynamics of follicular growth, CL size, and P4 concentrations in cyclic (n = 36) and anestrous (n = 30) Nelore cows. Cyclic (C) and anestrous (A) cows were divided into three groups. The control group received 2 mg of estradiol benzoate via intramuscular (IM) injection and an intravaginal device containing 1.9 g of P4 on Day 0. On Day 8, the device was removed, and the animals received 12.5 mg of dinoprost tromethamine IM. After 24 hours, the animals received 1 mg of estradiol benzoate IM. In the eCG group, cows received the same treatment described for the control group but also received 400 UI of eCG at the time of device removal. In the TCR group, calves were separated from the cows for 56 hours after device removal. Ultrasound exams were performed every 24 hours after device removal until the time of ovulation and 12 days after ovulation to measure the size of the CL. On the same day as the CL measurement, blood was collected to determine the plasma P4 level. Statistical analyses were performed with a significance level of $P \leq 0.05$. In cyclic cows, the presence of the CL at the beginning of protocol resulted in a smaller follicle diameter at the time of device removal (7.4 ± 0.3 mm in cows with CL vs. 8.9 ± 0.4 mm in cows without CL; $P = 0.03$). All cows ovulated within 72 hours after device removal. Anestrous cows treated with eCG or TCR showed follicle diameter at fixed-timed artificial insemination (A-eCG 10.2 ± 0.3 and A-TCR 10.3 ± 0.5 mm) and follicular growth rate (A-eCG 1.5 ± 0.2 and A-TCR 1.3 ± 0.1 mm/day) similar to cyclic cows (C-eCG 11.0 ± 0.6 and C-TCR 12.0 ± 0.5 mm) and (C-eCG 1.4 ± 0.2 and C-TCR 1.6 ± 0.2 mm/day, respectively; $P \leq 0.05$). Despite the similarities in CL size, the average P4 concentration was higher in the A-TCR (9.6 ± 1.4 ng/mL) than in the A-control (4.0 ± 1.0 ng/mL) and C-TCR (4.4 ± 1.0 ng/mL) groups ($P < 0.05$). From these results, we conclude that eCG treatment and TCR improved the fertility of anestrous cows by providing follicular growth rates and size of dominant follicles similar to cyclic cows. Additionally, TCR increases the plasma concentrations of P4 in anestrous cows.

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1. Introduction

Despite the clear benefits of artificial insemination (AI) for reproductive efficiency, this technique is not widely used in many tropical countries [1] because of biological and operational constraints. For example, the large size of some herds with thousands of cows under pasturing systems can hamper the use of AI because of an operational perspective. Furthermore, certain physiological traits, such as a short period of estrous behavior (11 hours) and a predominance of nightly manifestations of estrus, can result in failures to detect heat, resulting in low conception rates in the *Bos indicus* breeds [2]. Physiological postpartum anestrus, a condition that can be exacerbated by nutritional imbalances and climate conditions (dry season) and inhibited by suckling, is also problematic in these breeds [3].

Considering these challenges, fixed-timed artificial insemination (FTAI) represents the best solution to increase the use of AI in large *Bos indicus* herds. However, many aspects of FTAI require additional investigation, specifically the cyclicity, postpartum period, and weaning effects. Fixed-timed artificial insemination with progesterone (P4) has been used to induce cyclicity during the breeding season and markedly increasing conception rates [1,4]. The use of eCG and/or temporary calf removal (TCR) in conjunction with progestogen removal has led to conception rates of approximately 50% in noncyclic Nelore cows [5,6].

The use of eCG has been shown to result in an increase in the daily follicular growth rate, an increase in the diameter of the dominant follicle (DF), and higher ovulation rates in noncyclic indicus cows [7]. The controlled interruption of suckling has been used to increase GnRH levels and LH pulsatility, thereby improving ovulation in anestrous cows without hormone treatment [8]. However, the effects of eCG administration and TCR on follicular growth and luteum function in cyclic and anestrous Nelore cows have not been previously investigated.

The objective of this present study was to evaluate the effects of eCG and TCR on the follicular growth, CL size and P4 concentrations in cyclic and anestrous Nelore cows.

2. Materials and methods

2.1. Location, feed management, and animal selection

The experiments were conducted in Parana State, Brazil, 21°18′S, 52°49′W, during the summer in the Southern Hemisphere. This region has a tropical climate with a mean annual temperature of 22 °C and thermal amplitude of 7 °C. The rainy period lasts from November to March, and the annual precipitation ranges from 1000 to 1500 mm.

A total of 66 pluriparous Nelore cows (*Bos taurus indicus*) with average ages of 6.6 ± 2.1 years, 77 ± 12.9 days postpartum (suckling), and body condition score of 3 ± 0.4 (on a scale of 1–5 [9]) were used in this study. The cows were evaluated using transrectal palpation and ultrasonography, and the cyclicity was determined after two ultrasound exams (SSD 500 Aloka, Tokyo, Japan, with a 5.0 MHz linear transducer) of the ovaries performed 10 days apart. Cyclic cows (n = 36) presented a CL, whereas anestrous females (n = 30) had no CL.

During the experimental period, the animals were maintained by continuous grazing of *Brachiaria brizantha* with mineralized salt and water provided ad libitum.

2.2. Experimental design

Cyclic and anestrous cows (n = 66) were divided into six groups according to body condition score and postpartum period. Cyclic cows (n = 36) were allocated into three groups of 12 (cyclic control, cyclic eCG and cyclic TCR), whereas anestrous females (n = 30) were distributed into three groups of 10 (anestrous control, anestrous eCG, and anestrous TCR), as outlined in Figure 1.

In the cyclic control (C-con, n = 12) and anestrous control (A-con, n = 10) groups, animals received an intravaginal device containing 1.9 g of P4 (CIDR; Zoetis, Brazil) and an intramuscular (IM) injection of 2 mg of estradiol benzoate (EB) (Estrogen; Farmavet, Brazil) on Day 0. The device was removed on Day 8, and the cows received an IM injection of 12.5 mg of dinoprost tromethamine (Lutalyse; Zoetis, São Paulo, Brazil). After 24 hours, the animals received 1 mg of EB IM. In the cyclic eCG (C-eCG, n = 12) and anestrous eCG (A-eCG, n = 10) groups, cows received the same treatment as the control group but also received 400 IU of eCG IM (Novormon; Syntex, Buenos Aires, Argentina) at the time of device removal. The cyclic TCR (C-TCR, n = 12) and anestrous TCR (A-TCR, n = 10) groups received the same treatment as the control group, but TCR was also applied for 56 hours starting at the time of P4 device removal. The cows were kept approximately 2 km away from the calves to eliminate the possibility of contact by touch, sight, sound, or smell. The calves were kept on pasture with free access to water.

2.3. Ultrasonography and follicular dynamics

All animals were evaluated using ultrasonography every 24 hours to measure the DF diameter, CL diameter, and ovulation rate.

The size and location of the follicles and CL were documented for both ovaries and registered in individual maps for further monitoring. Follicles 5 mm or more in diameter were measured using ultrasonography and the diameter was calculated as the average of two cross-sectional linear measurements of the follicular antrum [10].

The DF was defined as that which grew to at least 8 mm and exceeded the diameter of all other follicles. Ovulation was monitored using ultrasonography every 24 hours after FTAI, detected by the absence of the previously identified DF and confirmed by the eventual presence of a CL in the same ovary [11]. At 12 days after ovulation, the CL was measured in the same ovary that previously contained the DF. The diameter was calculated as the average of two linear measurements of the cross-sectional surface of the CL [10]. The follicular growth rate of within each cow was determined by measuring the difference in the follicle size over 24-hour increments of time [11]. Additionally, cyclic animals were evaluated using ultrasonography on Day 8