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Evaluation of the hypothalamus–pituitary axis response to exogenous GnRH, estradiol benzoate, and LH during the postpartum period in Nellore cows

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ABSTRACT

The objective was to evaluate when the LH reserve was re-established in postpartum Nellore (*Bos indicus*) cows by evaluating the response of the hypothalamic–pituitary axis responsiveness to exogenous GnRH or estradiol benzoate (EB). Additionally, we tested the influence of dietary supplementation (SUPL) and calf removal (CR) on the duration of postpartum anestrus. Ninety multiparous lactating Nellore cows were randomly assigned to eight groups. The EB and GnRH groups received 1.0 mg EB (N = 7), and 50 μg lecireline (N = 16), respectively. Additional cows were given the same hormones, and subjected to either nutritional supplementation (EB-SUPL, N = 9; GnRH-SUPL, N = 16), or calf removal at 72 hours after calving (EB-CR, N = 4; GnRH-CR, N = 13). The remaining two groups were the LH (12.5 mg, N = 14) and control groups (saline, N = 11). Hormones were administered weekly from 7 (±5) days postpartum to first ovulation (detection of a CL during a weekly ultrasonographic examination). Blood samples were collected just before and 2 hours (GnRH, LH, and control groups) or 18 hours (EB groups) after hormone or saline (control) administration. Ovulation occurred as early as 15 days postpartum in the GnRH group. The mean ± SEM intervals (days) from calving to first ovulation were EB, 87.7 ± 4.2; EB-CR, 20.3 ± 1.2; EB-SUPL, 60.3 ± 3.2; GnRH, 40.4 ± 2.1; GnRH-CR, 21.0 ± 1.1; GnRH-SUPL, 26.4 ± 1.1; LH, 35.6 ± 1.1; and control, 60.9 ± 2.1. We concluded that there was sufficient LH in the pituitary gland (of Nellore cows) from the second week postpartum to induce ovulation in response to exogenous GnRH. Additionally, calf removal and nutritional supplementation reduced, by 2 to 4 weeks, the interval from calving to an LH increase and ovulation induced by GnRH or EB.

1. Introduction

A limiting factor in the beef cattle industry is prolonged postpartum anestrus, particularly in cattle in a pasture-based management system. This period is necessary for uterine involution and re-establishment of the activity of the hypothalamus–pituitary–gonad–uterus axis [1,2]. This period lasts from calving to first signs of behavioral estrus [2].

Because GnRH secretion is not completely suppressed, premature resumption of FSH pulses can occur because of the low frequency of GnRH. However, LH pulses might restart later in the postpartum period, but only when the GnRH pulses increase [3]. The pulsatile pattern of LH release in the postpartum period is characterized by low frequency (less than one pulse per 4 hours [4]). However, this frequency increases during the period immediately before ovulation (up to one LH pulse per 1 or 2 hours [5–7]).

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Numerous factors can influence LH secretion and ovarian function in the postpartum period; the most important include body condition score, nutritional condition [8], energy balance [9], and frequency at which the calf suckles [3].

The development of the first dominant follicle usually occurs 10 to 12 days postpartum in beef and dairy cows [6,10–13]. In suckled beef cows, cyclicity is re-established between 35 to 60 days postpartum when the animals are able to maintain a satisfactory body condition score. However, it might be delayed if the body condition score is not favorable [7,14]. In most cases, the first postpartum ovulation occurs without behavioral estrus [10,11,15] because of the limited capacity of the dominant follicle to produce estradiol during postpartum anestrus [16]. Although these events are well described in *Bos taurus* cattle, the reproductive physiology of *Bos indicus* cattle [13,17] is not as well characterized.

The objective of the present study was to evaluate when the LH reserve was re-established in postpartum Nellore (*Bos indicus)* cows by evaluating the response of the hypothalamic–pituitary axis responsiveness to exogenous GnRH or estradiol benzoate (EB). Additionally, we tested the influence of dietary supplementation (SUPL) and calf removal (CR) on the duration of postpartum anestrus in Nellore cows (*Bos indicus*).

2. Materials and methods

All experimental cows were managed according to the guidelines approved by the Ethics Committee of the School of Veterinary Medicine at the São Paulo State University.

2.1. Cows and feeding

The experiment was conducted at the Yguapora Farm located in São Paulo state, Brazil (23°19′S; 49°23′W), from January to March of 2009 and January to March of 2010. Ninety lactating multiparous Nellore cows were housed in a *Brachiaria brizantha* grass pasture with *ad libidum* access to minerals and water. Nutritional supplementation offered to the EB-SUPL and GnRH-SUPL groups was balanced according to the National Research Council [18]. This supplementation consisted of 60% ground corn and 40% cottonseed meal (20.5% crude protein and 78% total digestible nutrients) and was offered from immediately after calving to 1 week after ovulation. The cows were weighed and their body condition score (five-point scale [19]) was evaluated 45 days before the beginning of the experiment, the day that the experiment started, and weekly throughout the experiment.

2.2. Treatments

During Year 1 of the experiment (2009), cows were randomly divided into eight groups according to treatment groups: (1) group EB: the animals received 1 mg EB (im, Estrogin; Farmavet, São Paulo, Brazil) (N = 7); (2) group EB-CR was similar to the EB group, however, the calf was removed 72 hours after calving (N = 4); (3) group EB-SUPL was similar to the EB group, however, the diet was supplemented with 3.5 kg per cow per day of ground corn and cottonseed meal (N = 9); (4) in the GnRH group, cows received 50 μg lecireline im (Gestran Plus; ARSA S.L.R., Buenos Aires, Argentina) (N = 8); (5) group GnRH-CR was similar to the GnRH group, except that calves were removed 72 hours after calving (N = 6); (6) group GnRH-SUPL was similar to the GnRH group, except the diet was supplemented with 3.5 kg per cow per day of ground corn and cottonseed meal (N = 8); (7) in the LH group, cows received 12.5 mg LH (im, Lutropin, Bioniche Animal Health, Belts-ville, Ontario, Canada) (N = 7); and (8) the control group received saline (N = 5). During Year 2 (2010), the estradiol benzoate treatment group and the related groups (CR and SUPL) were removed from the experiment and the animals were randomly distributed into five groups: (1) GnRH (N = 8); (2) GnRH-CR (N = 7); (3) GnRH-SUPL (N = 8); (4) LH (N = 7); and (5) control group (N = 6), as described above.

Hormones were administered from 7 ± 5 days postpartum to detection of first ovulation, based on once-weekly examinations with transrectal ultrasonography (Aloka 900 with 7.5 MHz, linear-array transducer, Aloka, Tokyo, Japan). In that regard, the absence of a dominant follicle (previously detected by ultrasonography), and the subsequent presence of luteal tissue was defined as ovulation. Furthermore, ovulation data were supported by plasma progesterone concentrations, which were greater than 1 ng/mL, or at least 50% greater than the initial concentration (before hormone treatment) in 49% of cows. Ovulation was designated to have occurred 1 day after hormone treatment.

Cows were subjected to two blood draws weekly: (1) immediately before treatment (GnRH, LH, EB, or saline); and (2) 2 hours after injection of GnRH, LH, or saline, or 18 hours after EB, to determine concentrations of LH and progesterone by radioimmunoassay. The timing of blood collection was based on the literature, which indicates that the LH surge occurs 2 and 18 hours after exogenous GnRH [20] and EB [21], respectively. The hormonal treatment scheme is summarized (Fig. 1).

2.3. Radioimmunoassay

Blood (approximately 10 mL) was collected from the jugular vein into tubes containing heparin (BD Vacutainer; Franklin Lakes, NJ, USA). After the blood was drawn, samples were immediately stored on ice. Plasma was separated by centrifugation (900× *g* for 20 minutes at approximately 25 °C) and stored at −20 °C until analysis.

Plasma progesterone concentration was determined according to radioimmunoassays previously described [22,23]. The intra- and interassay coefficients of variation were 4.82% and 10.61%, respectively.

Plasma LH concentrations were determined as described [24]. The intra- and interassay means of the coefficients of variation were 7.79% and 7.88%, respectively. The basal concentration levels of LH in the control group were 1.65 ± 0.2 ng/mL. For each cow, the threshold for an LH increase was defined as an increase in the LH concentration mean plus at least two-fold of the SD from the concentration of the first LH measurement before hormone treatment (GnRH, LH, or EB).