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Different circulating progesterone concentrations during synchronization of ovulation protocol did not affect ovarian follicular and pregnancy responses in seasonal anestrous buffalo cows

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ABSTRACT

Three experiments were designed to evaluate the effect of different circulating progesterone (P4) concentrations during synchronization of ovulation protocol for timed artificial insemination of seasonal anestrous buffalo cows. In the first trial, ovariectomized cows were randomly allocated into one of three groups: using new P4 devices (G-New; n = 8), using devices previously used for 9 days (G-Used1x; n = 8), and using devices previously used for 18 days (G-Used2x; n = 8). The P4 device was maintained for 9 days, and the circulating P4 concentration was measured daily. The circulating P4 concentrations during the P4 device treatment were the lowest for G-Used2x (1.10 ± 0.04 ng/mL), intermediate for G-Used1x (1.52 ± 0.05 ng/mL), and the highest for G-New (2.47 ± 0.07 ng/mL; P = 0.001). In the second trial, 31 anestrous cows had their ovarian follicular dynamics evaluated after receiving the treatments described previously (G-New [n = 10], G-Used1x [n = 11], and G-Used2x [n = 10]). At insertion of the P4 device, cows were administered 2.0 mg of estradiol benzoate. Nine days later, the P4 device was removed and cows were administered 0.53 mg of cloprostenol sodium plus 400 IU of eCG. Forty-eight hours after P4 device removal, 10 μg of buserelin acetate was administered. There were no differences among the groups (G-New vs. G-Used1x vs. G-Used2x) in diameter of the largest follicle at P4 device removal (9.0 ± 0.8 vs. 10.1 ± 0.9 vs. 8.6 ± 0.8 mm; P = 0.35), in interval from P4 device removal to ovulation (77.1 ± 4.5 vs. 76.5 ± 4.7 vs. 74.0 ± 4.4 hours; P = 0.31), or in ovulation rate (80.0% vs. 81.8% vs. 60.0%; P = 0.51). In experiment 3, 350 anestrous cows were randomly assigned into one of the three treatments described previously (G-New, n = 111; G-Used1x, n = 121; G-Used2x, n = 118) and received a timed artificial insemination for 16 hours after buserelin treatment. The 30-day pregnancy rates did not differ among groups (55.9% vs. 55.4% vs. 48.3%; P = 0.39). Thus, the low circulating P4 concentrations released from a used P4 device efficiently control the ovarian follicular growth and had no detrimental effect on the pregnancy rates of the seasonal anestrous buffalo cows.

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1. Introduction

Hormonal treatments have been designed to control luteal and follicular functions, providing exciting possibilities for synchronization of ovulation to enable the use of timed artificial insemination (TAI) in buffaloes [1–8]. This breeding strategy has been efficiently applied during and out of the breeding season, regardless of the cyclic status at the onset of the synchronization of ovulation protocol [1,5,9,10].

The estradiol plus progesterone (P4)-based TAI protocol has been the most used TAI synchronization protocol in buffaloes out of the breeding season [1]. In seasonal anestrous buffaloes, a common feature of the synchronization of ovulation protocols for TAI is the insertion of an intravaginal device containing P4 plus administration of estradiol benzoate (2.0 mg intramuscular; Sincrodiol; Ourofino Agronegócio, São Paulo, Brazil) on Day 0, and an injection of PGF2α on Day 9, at the time of device withdrawal, plus 400 IU of eCG (Novormon; MSD Animal Health, Sao Paulo, Brazil). The GnRH is used as an inducer of ovulation and is administered 48 hours after removal of the P4 device. The TAI is generally performed 16 hours after the GnRH treatment [1,4,7].

In cattle, the P4 milieu during the synchronization of ovulation protocol for TAI influences the reproductive outcome by altering the health of the ovulatory follicle and thus altering the likelihood of pregnancy [11,12]. Many researchers have found that the circulating P4 concentrations are able to control the LH pulsatility [13,14], final dominant follicle growth, and ovulation response [15,16] in cattle. However, in high-producing dairy cows, low circulating P4 concentration during the preovulatory period can negatively alter several reproductive processes associated with oocyte quality and the establishment of pregnancy [17–20], affecting the uterine morphology and secretory functions [21–23] and thereby leading to shortened subsequent CL lifespan [21]. Conversely, beef cows treated with a subluteal phase P4 milieu during ovulatory follicular growth induced follicles to grow to a larger size, resulting in higher pregnancy rates than cows with a luteal P4 milieu [24]. Nevertheless, the minimum plasmatic P4 concentrations required to improve fertility in buffalo cattle lacking a CL at the initiation of the TAI program remain unknown. Furthermore, it could be expected that seasonal anestrous lactating buffaloes created under tropical grazing conditions are more similar to suckled beef than high-producing dairy cows in terms of the minimal P4 levels requirement during synchronization of ovulation protocol.

Thus, the aim of the present study was to evaluate the effect of different circulating P4 concentrations during a synchronization of ovulation protocol for TAI in seasonal anestrous buffalo cows. The present hypothesis is that the lower circulating P4 concentration released from a reused P4 device treatment is effective to control ovarian follicular growth and had no detrimental effect on pregnancy results in seasonal anestrous buffalo cows subjected to an estradiol plus P4-based synchronization of ovulation protocol for TAI.

2. Materials and methods

2.1. Experiment 1—Circulating P4 profiles in ovariectomized buffalo cows

2.1.1. Animal and management

This experiment was conducted on a state research farm (Unidade de Pesquisa e Desenvolvimento de Registro, São Paulo, Brazil) during the out of breeding season (spring to summer in the southern hemisphere; November to March). A total of 24 ovariectomized buffalo cows, 66.0 ± 8.4 (mean ± standard error of the mean) months old, presenting a body condition score (BCS) of 3.7 ± 0.1 (scale 1–5, where 1 = very thin and 5 = very fat) and weighing 568.7 ± 15.9 kg at the first day of the trial were used. Cows had their ovaries surgically removed (via laparotomy) at least 1 year before the beginning of the present experiment. Buffaloes were maintained on a *Brachiaria decumbens* pasture with free access to water and mineralized salt.

2.1.2. Experimental design

Ovariectomized buffaloes were randomly allocated into one of three groups using different types of intravaginal P4 devices (Sincrogest; Ourofino Agronegócio): new device (1.0 g of P4; G-New; n = 8), previously used for 9 days (G-Used1x; n = 8), and previously used for 18 days (G-Used2x; n = 8). All buffaloes were treated at the same time and the devices from G-Used1x and G-Used2x were used from previous synchronizations. The P4 device was maintained for 9 days. After initial use, the P4 devices were individually washed with water and then soaked in a solution of chloride alkyl dimethyl benzyl ammonium (CB 30; Ourofino Agronegócio) for approximately 10 minutes. Thereafter, the P4 devices were dried using brown paper, placed inside aluminum bags, and stored at room temperature until use.

2.1.3. Blood sampling and P4 assay

Blood samples were collected by jugular venipuncture for quantifying the daily circulating P4 concentration over the 9 days of use of the P4 device. Blood samples were collected using evacuated tubes. Samples were kept at room temperature and transported to the laboratory within 4 hours of collection. Blood tubes were centrifuged at ×3000*g* for 20 minutes, and serum was frozen at −25 °C until analysis. The serum P4 concentrations were evaluated from unextracted sera using an antibody coated-tube RIA kit (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA, USA), previously validated by Garbino, et al. [23]. The intra-assay coefficient of variation was 2.6%, and the assay sensitivity was 0.006 ng/mL. The low and high interassay coefficient variations were 6.4% and 0.7%, respectively.

2.2. Experiment 2—Ovarian follicular dynamics of seasonal anestrous buffalo cows

2.2.1. Animals and management

This experiment was conducted in the same state research farm cited in experiment 1, out of the breeding season (spring to summer in the southern hemisphere;