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Resynchronization with unknown pregnancy status using progestin-based timed artificial insemination protocol in beef cattle

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ABSTRACT

Two experiments were designed to evaluate the use of resynchronization (RESYNCH) protocols using a progestin-based timed artificial insemination (TAI) protocol in beef cattle. In experiment 1, 475 cyclic Nelore heifers were resynchronized 22 days after the first TAI using two different inducers of new follicular wave emergence (estradiol benzoate [EB; n = 241] or GnRH [n = 234]) with the insertion of a norgestomet ear implant. At ear implant removal (7 days later), a pregnancy test was performed, and nonpregnant heifers received a dose of prostaglandin plus 0.5 mg of estradiol cypionate, with a timed insemination 48 hours later. The pregnancy rate after the first TAI was similar (P = 0.97) between treatments (EB [41.9%] vs. GnRH [41.5%]). However, EB-treated heifers (49.3%) had a greater (P = 0.04) pregnancy per AI (P/AI) after the resynchronization than the GnRH-treated heifers (37.2%). In experiment 2, the pregnancy loss in 664 zebu females (344 nonlactating cows and 320 cyclic heifers) between 30 and 60 days after resynchronization was evaluated. Females were randomly assigned to one of two groups (RESYNCH 22 days after the first TAI [n = 317] or submitted only to natural mating [NM; n = 347]). Females from the NM group were maintained with bulls from 15 to 30 days after the first TAI. The RESYNC-treated females were resynchronized 22 days after the first TAI using 1 mg of EB on the first day of the resynchronization, similar to experiment 1. No difference was found in P/AI (NM [57.1%] vs. RESYNC [61.5%]; P = 0.32) or pregnancy loss (NM [2.0%] vs. RESYNC [4.1%]; P = 0.21) after the first TAI. Moreover, the overall P/AI after the RESYNCH protocol was 47.5%. Thus, the administration of 1 mg of EB on day 22 after the first TAI, when the pregnancy status was undetermined, promotes a higher P/AI in the resynchronized TAI than the use of GnRH. Also, the administration of 1 mg of EB 22 days after the TAI did not affect the preestablished pregnancy.

1. Introduction

The incorporation of biotechnologies, such as artificial insemination (AI), to improve the genetic gains in calf operation systems is essential. To accomplish these genetic gains, the reproductive programs must focus on enhancing the proportion of cows calving from AI by improving the service rates and reducing the interval between inseminations without compromising the survival of the embryo/fetus.

Tropical countries have a significant participation in the world's beef production. Zebu cattle are the main breed raised in these areas. Furthermore, there are some reproductive physiologic differences between *Bos taurus* and

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B indicus that should be considered in establishing reproductive management programs [1–3]. Among these differences, the short length of estrus and the high incidence of nocturne estrus could significantly affect AI programs based on the detection of estrus [4,5]. Despite the satisfactory efficiency of the GnRH treatment as ovulatory stimulus at the end of TAI protocol in *B indicus* cattle [6], the breed presents reduced response to the GnRH plus prostaglandin $(PGF)_{2\alpha}$-based TAI protocols [7,8] compared with *B taurus* cattle [1,9]. As result of this lower response, progestin/progesterone (P4) plus estradiol (E2)-based TAI protocols have been the most common type of commercially used timed synchronization protocol in tropical countries [10–13].

Previous studies have demonstrated that is possible to resynchronize the return of estrus of in nonpregnant cows after the first AI in *B taurus* beef and dairy cattle [14–19]. To avoid the necessity of estrus detection for the second service, studies have proposed the initialization of the resynchronization (RESYNCH) protocol for the second TAI at the time of pregnancy diagnosis. Nonetheless, alternatively, another possibility may be to perform the onset of the RESYNCH protocol before the pregnancy examination, while the pregnancy status is still unknown, so that the final resynchronization treatment (i.e., $PGF_{2\alpha}$) is performed immediately after a nonpregnant diagnosis [19–24]. These strategies are effective in reducing the interval between the first and second inseminations [19,20]. Furthermore, treatments with GnRH on Day 21 after AI, when the pregnancy status was still undetermined, had no detrimental effect on the pregnancy rate of the previous AI in dairy cows [17,20,22].

Most studies that resynchronized females with an unknown pregnancy status for a second TAI initiated hormone therapy with the GnRH treatment to induce new follicular wave emergence at the onset of the RESYNCH protocol. However, limited information is available regarding the use of E2 at the beginning of the RESYNCH protocol, with an unknown pregnancy status, for the second TAI. Previous studies demonstrated that E2 given in the middle of the estrous cycle (13–14 days after AI) of dairy and beef cattle for resynchronization of the return to estrus could compromise the ability of the CL to produce P4 [15,25] and reduce the preestablished pregnancy in beef heifers [18,26]. In dairy cattle, one study reported the use of E2 at the beginning of the RESYNCH protocol, 23 days after the first TAI. This protocol resulted in a satisfactory conception rate after the second AI [16]. However, the authors did not provide information on whether the estradiol benzoate (EB) administration had any negative effect on the preestablished pregnancy at the onset of the RESYNCH protocol [16].

Therefore, the present study evaluated (1) the use of two inducers of new follicular wave emergence (GnRH vs. EB) on pregnancy per AI (P/AI) after the RESYNCH protocol initiated with an unknown pregnancy status in cyclic beef heifers and (2) the influence of the administration of 1 mg of EB on the first day of the RESYNCH protocol (i.e., 22 days after the first TAI) on pregnancy loss within 30 to 60 days of a preestablished pregnancy. Our hypothesis was that the administration of 1 mg of EB on Day 22 after the first TAI, when the pregnancy status was undetermined, promotes a higher P/AI in the resynchronized TAI. However, the administration of 1 mg of EB 22 days after the first TAI may have a detrimental effect on a preestablished pregnancy in zebu beef cattle.

2. Materials and methods

2.1. Experiment 1

2.1.1. Animals and management

This experiment was conducted during the 2010/2011 spring–summer breeding season. A total of 475 cyclic (the presence of CL on the first day of the breeding season) Nelore (*B indicus*) heifers allocated in five different breeding groups (i.e., pastures) from a commercial beef farm in the state of Mato Grosso, Brazil, were used. Heifers with unknown pregnancy status were enrolled 22 days after the previous TAI. All heifers were maintained on *Brachiaria decumbens* pastures, with free access to water and mineralized salt. At the beginning of the first TAI protocol, data on the age and the body condition score (BCS; range, 1 [emaciated] to 5 [obese] [27]) were collected on each heifer. For purpose of analyses of the relationships between BCS and P/AI, heifers were classified according to BCS on the first day of the first TAI synchronization protocol as having lesser (BCS $\leq$ 3.00), moderate (BCS = 3.25), or greater (BCS $\geq$ 3.50) BCS.

2.1.2. Reproductive management and treatments

At the onset of the breeding season, heifers presenting a CL were subjected to an E2 plus norgestomet ear implant-based TAI protocol for the first TAI according to previous study [6]. At the initiation of the synchronization protocol, heifers received a new norgestomet ear implant (Crestar, MSD Animal Health, São Paulo, Brazil) in combination with 2 mg im of EB (Estrogin, Farmavet, São Paulo, Brazil). Eight days later (Day 8), the ear implants were removed, and all heifers received intramuscularly 150 mg of D-cloprostenol (Preloban, MSD Animal Health), 300 IU of equine chorionic gonadotropin (eCG, Folligon, MSD Animal Health), and 0.5 mg of estradiol cipionate (ECP, Zoetis, São Paulo, Brazil). All females were inseminated 48 hours after removal of the ear implant (Fig. 1).

Twenty-two days after the first TAI (Day 22), heifers with an unknown pregnancy status were randomly assigned to one of two experimental groups, either receiving 1 mg of EB (n = 241) or 100 μg of gonadorelin (n = 234; GnRH; Fertagyl, MSD Animal Health) to induce a new follicular wave emergence. At the same time, heifers received a NORG implant that had previously been used for 8 days. According to previous data, similar response was expected regarding the use of new (first TAI) or used NORG (second TAI) ear implant in *B indicus* cattle [6,28]. After their initial use, the NORG ear implants were individually washed with water and then soaked in a solution of ammonium chloride (CB 30, Ouro fino Agronegocio, São Paulo, Brazil) for approximately 10 minutes. Thereafter, the implants were dried using brown paper, thoroughly wrapped in aluminum paper, and stored at room temperature until use. On Day 29 (7 days after the initial RESYNCH treatment), a pregnancy diagnosis was performed. Nonpregnant heifers received 150 mg of D-cloprostenol and 0.5 mg of ECP at the time the ear implant was removed, and TAI was performed 48 hours later. Frozen-