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Effect of progesterone concentrations, follicle diameter, timing of artificial insemination, and ovulatory stimulus on pregnancy rate to synchronized artificial insemination in postpubertal Nellore heifers

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ABSTRACT

Two experiments were designed to evaluate the effects of treatments with low versus high serum progesterone (P_4) concentrations on factors associated with pregnancy success in postpubertal Nellore heifers submitted to either conventional or fixed timed artificial insemination (FTAI). Heifers were synchronized with a new controlled internal drug release device (CIDR; 1.9 g of P_4 [CIDR1]) or a CIDR previously used for 18 days (CIDR3) plus 2 mg of estradiol (E_2) benzoate on Day 0 and 12.5 mg of prostaglandin F2α on Day 7. In experiment 1 ($n = 723$), CIDR were removed on Day 7 or 9 and heifers were inseminated after estrus detection. In experiment 2 ($n = 1083$), CIDR were all removed on Day 9 and FTAI was performed either 48 hours later in heifers that received E_2 cypionate (ECP) on Day 9 (0.5 mg; E48) or 54 or 72 hours later in conjunction with administration of GnRH (100 μg; G54 or G72). Synchronization with CIDR1 resulted in greater serum P_4 concentrations and smaller follicle diameters on Days 7 and 9 in both experiments. In experiment 1, treatment with CIDR for 9 days decreased the interval from CIDR removal to estrus (Day 7, 3.76 ± 0.08 days vs. Day 9, 2.90 ± 0.07; $P < 0.01$) and improved conception (Day 7, 57.1% vs. Day 9, 65.8%; $P = 0.05$) and pregnancy rates (Day 7, 37.6% vs. Day 9, 45.3%; $P = 0.04$). In experiment 2, treatment with ECP improved ($P < 0.01$) the proportion of heifers in estrus (E48, 40.9%[a]; G54, 17.1%[c]; and G72, 32.0%[b]), but the pregnancy rate was not affected ($P = 0.64$) by treatments (E48, 38.8%; G54, 35.5%; G72, 37.5%). Synchronization with CIDR3 increased follicle diameter at FTAI (CIDR1, 11.07 ± 0.10 vs. CIDR3, 11.61 ± 0.10 mm; $P < 0.01$), ovulation rate (CIDR1, 82.8% vs. CIDR3, 88.0%; $P < 0.01$) and did not affect conception (CIDR1, 42.2 vs. CIDR3, 45.1%; $P = 0.38$) or pregnancy rates (CIDR1, 34.7 vs. CIDR3, 39.4%; $P = 0.11$). In conclusion, length of treatment with P_4 affected the fertility of heifers bred based on estrus detection. When the heifers were submitted to FTAI protocol, follicle diameter at FTAI (≤10.7 mm, 23.6%; 10.8–15.7 mm, 51.5%; ≥15.8 mm, 30.0%; $P < 0.01$) was the main factor that affected conception and pregnancy rates.

1. Introduction

Previous reports have associated lower pregnancy rates at fixed timed artificial insemination (FTAI) in postpubertal

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Nellore heifers and either nonlactating or lactating cycling cows with elevated circulating concentrations of progesterone (P_4), during synchronization of ovulation protocols [1,2].

It has been suggested that high circulating concentrations of P_4 result in reduced LH pulse frequency, which decreases dominant follicle development [3,4] and subsequent follicle diameter at FTAI [2,5], compromising fertility [6–8]. Alternatives for increasing gonadotropic support of dominant follicles before FTAI may increase fertility in beef cattle submitted to a FTAI protocol [2,5,9]. These alternatives include the reduction in circulating concentration of P_4 by administrating a controlled internal drug release device (CIDR) previously used for 18 days [2,5], stimulation of endogenous gonadotropins release by treatment with prostaglandin (PG)F2α 2 days before CIDR removal [5,9], and administration of exogenous gonadotropin [2,5]. The CIDR containing 1.9 g of P_4 has been used successfully in estrous synchronization protocols as many as four times [9], and previous studies with postpubertal heifers demonstrated lower serum P_4 concentrations [2,5] and greater pregnancy rates after synchronization with a CIDR previously used for 18 days [2] compared with the protocol with a new CIDR.

An increased conception rate was associated with increased preovulatory concentrations of estradiol (E_2) before FTAI by increasing the length of proestrus [10,11] or administration of exogenous E_2 (estradiol cypionate [ECP]) [12]. In Brazil, ECP has been used as an ovulatory stimulus concurrent with progestin removal in synchronization of ovulation protocols [1,2,9]. Recently, Sá Filho, et al. [13] demonstrated that administration of ECP at progestin removal increased the proportion of Nellore cows showing estrus and increased pregnancy rate compared with cows treated with GnRH.

Therefore, reduced circulating P_4 concentrations [2,5] during synchronization of ovulation protocols and increasing preovulatory E_2 before FTAI [10,11,13], could result in improvements in fertility. Thus, this study was designed to evaluate the effects of P_4 concentrations and duration of P_4 treatment on follicle development during estrous synchronization protocols, and their impacts on fertility of postpubertal Nellore heifers. In the first study (experiment 1), the effects of P_4 concentrations and length of P_4 treatment on follicle diameter, estrus distribution, and conception rate to AI based on estrus were evaluated. To evaluate possible effects of increased preovulatory concentrations of E_2 before FTAI, the second study (experiment 2) focused on comparing the impact of the ovulatory stimulus ECP or GnRH (at 54 or 72 hours) on pregnancy rates.

2. Material and methods

Experiments were conducted in commercial beef farms located in Mato Grosso, Brazil. Heifers were maintained on pastures (*Brachiaria brizantha*) with water and mineral salt *ad libitum*. Only heifers with a CL on one of two ultrasonography evaluations (Aloka SSD-500 with a 7.5 MHz linear-array transrectal transducer; Aloka, Tokyo, Japan) performed within an interval of 10 days between 30 and 7 days before the initiation of protocols were included in these studies. All animals were cared for in accordance with the practices outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching [14].

2.1. Experiment 1

The goals of this experiment were to evaluate the effect of CIDR (new vs. previously used for 18 days) and time of CIDR treatment (7 vs. 9 days) on follicle development and fertility of postpubertal Nellore heifers submitted to AI after estrus detection.

Postpubertal Nellore heifers (n = 723; body condition score [BCS] of 3.01 ± 0.24 on Day 0 using a scale from 1 = emaciated to 5 = obese [15]; 24–29 months of age) were allocated in six pastures, and synchronized between January and March 2010. Heifers were randomly assigned within each pasture to receive either a new P_4-releasing intravaginal device containing 1.9 g P_4 (CIDR; Pfizer Animal Health, São Paulo, SP, Brazil, first use; CIDR1) or a CIDR that had been previously inserted in two 9-days synchronization protocols (third use; CIDR3). All heifers were treated with 2 mg of estradiol benzoate im (2.0 mL Estrogin; Farmavet, São Paulo, SP, Brazil) at CIDR insertion (Day 0) and 12.5 mg im dinoprost tromethamine (PGF2α; 2.5 mL Lutalyse; Pfizer Animal Health) on Day 7. The CIDRs were removed on either Day 7 (d7) or 9 (d9) within the CIDR1 and CIDR3 groups, resulting in treatments designated as CIDR1-d7 (n = 175), CIDR1-d9 (n = 189), CIDR3-d7 (n = 166), and CIDR3-d9 (n = 193). In a subgroup of heifers (n = 408) from CIDR1-d7 (n = 96), CIDR1-d9 (n = 87), CIDR3-d7 (n = 111), and CIDR3-d9 (n= 114), ovaries were evaluated on Days 7 and 9 by a single technician using ultrasonography to assess the diameter of the largest ovarian follicle (defined as the average between horizontal and vertical diameters). In the same subgroup, blood samples were collected for subsequent P_4 analyses. After CIDR removal, visual estrous detection occurred twice daily for 1 hour (0730 and 1600 hours) during 7 days (Days 8–14 in CIDR1-d7 and CIDR3-d7; Days 10–16 in CIDR1-d9 and CIDR3-d9 treatments). Heifers observed in estrus were inseminated by a single AI technician 10 to 12 hours later, with frozen–thawed commercial semen of two bulls that were randomly assigned to treatments. Immediately before insemination, the same technician determined the diameter of the largest follicle of all heifers in estrus by transrectal ultrasonography. Pregnancy diagnosis was based on detection (transrectal ultrasonography) of an embryo 40 days after the end of estrus detection period. Reproductive variables that were determined included estrus detection, conception, and pregnancy rates. Estrus detection rate was calculated as the proportion of all heifers treated that were observed in estrus. Conception rate was calculated as the proportion of heifers observed in estrus that became pregnant to AI. Pregnancy rate was calculated as the proportion of all heifers initially treated that became pregnant to AI.

2.2. Experiment 2

The goals of this experiment were to evaluate the effect of administration of ECP or GnRH (54 or 72 hours after CIDR