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Increasing length of an estradiol and progesterone timed artificial insemination protocol decreases pregnancy losses in lactating dairy cows

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ABSTRACT

Our hypothesis was that increasing the length of an estradiol and progesterone (P4) timed artificial insemination (TAI) protocol would improve pregnancy per artificial insemination (P/AI). Lactating Holstein cows (n = 759) yielding 31 ± 0.30 kg of milk/d with a detectable corpus luteum (CL) at d −11 were randomly assigned to receive TAI (d 0) following 1 of 2 treatments: (8d) d −10 = controlled internal drug release (CIDR) and 2.0 mg of estradiol benzoate, d −3 = $PGF_{2\alpha}$ (25 mg of dinoprost tromethamine), d −2 = CIDR removal and 1.0 mg of estradiol cypionate, d 0 = TAI; or (9d) d −11 = CIDR and estradiol benzoate, d −4 = $PGF_{2\alpha}$, d −2 CIDR removal and estradiol cypionate, d 0 TAI. Cows were considered to have their estrous cycle synchronized in response to the protocol by the absence of a CL at artificial insemination (d 0) and presence of a CL on d 7. Pregnancy diagnoses were performed on d 32 and 60. The ovulatory follicle diameter at TAI (d 0) did not differ between treatments (14.7 ± 0.39 vs. 15.0 ± 0.40 mm for 8 and 9 d, respectively). The 9d cows tended to have greater P4 concentrations on d 7 in synchronized cows (3.14 ± 0.18 ng/mL) than the 8d cows (3.05 ± 0.18 ng/mL). Although the P/AI at d 32 [45 (175/385) vs. 43.9% (166/374) for 8d and 9d, respectively] and 60 [38.1 (150/385) vs. 40.4% (154/374) for 8d and 9d, respectively] was not different, the 9d cows had lower pregnancy losses [7.6% (12/166)] than 8d cows [14.7% (25/175)]. The cows in the 9d program were more likely to be detected in estrus [72.0% (269/374)] compared with 8d cows [62% (240/385)]. Expression of estrus improved synchronization [97.4 (489/501) vs. 81% (202/248)], P4 concentrations at d 7 (3.22 ± 0.16 vs. 2.77 ± 0.17 ng/mL), P/AI at d 32 [51.2 (252/489) vs. 39.4% (81/202)], P/AI at d 60 [46.3 (230/489) vs. 31.1% (66/202)], and decreased pregnancy loss [9.3 (22/252) vs. 19.8% (15/81)] compared with cows that did not show estrus, respectively. Cows not detected in estrus with small (<11 mm) or large follicles (>17 mm) had greater pregnancy loss; however, in cows detected in estrus, no effect of follicle diameter on pregnancy loss was observed. In conclusion, increasing the length of the protocol for TAI increased the percentage of cows detected in estrus and decreased pregnancy loss.
Key words: length of the protocol, estrus, pregnancy loss

INTRODUCTION

Programs for estrous cycle synchronization have been used for reproductive management in many parts of the world. These programs involve a series of hormonal treatments designed to synchronize a follicular wave, synchronize corpus luteum (**CL**) function, and, finally, synchronize ovulation, allowing timed AI (**TAI**) without the need for detection of estrus (Pursley et al., 1997). Various hormones and timing of protocols have been used, with the most widely used programs using either estradiol (**E2**) or GnRH at the beginning of the protocol to synchronize emergence of a new follicular wave and E2 or GnRH at the end of the protocol to synchronize ovulation near TAI (Vasconcelos et al., 2011a; Wiltbank et al., 2011a).

Several studies have evaluated the effect of altering the time from initiation of CL regression until TAI on fertility during estrous cycle synchronization programs in beef (Meneghetti et al., 2009; Peres et al., 2009; Bridges et al., 2010) and dairy cattle (Peters and Pursley, 2003; Ribeiro et al., 2012; Pereira et al., 2013a). For example, in dairy cows, a significant ($P < 0.01$) linear trend of an increasing percentage of cows pregnant per AI (**P/AI**) with increasing time from treatment with prostaglandin $F_{2\alpha}$ (**PGF**), to induce CL regression, until treatment with GnRH, to induce synchronized ovulation (8.8% at 0 h; 13.2% at 12 h; 21.4% at 24 h; 28.0% at 36 h) was observed. In *Bos taurus* beef cattle, a summary of results from various studies done in the laboratory of Michael Day (Bridges et al., 2010) sug-

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gested that fertility increased as time from $PGF_{2\alpha}$ until GnRH increased. Similarly, in *Bos indicus* beef cattle synchronized with E2 and progesterone (**P4**), treatment with $PGF_{2\alpha}$ 4 d before TAI produced an increase in P/AI compared with $PGF_{2\alpha}$ treatment 2 d before TAI [time of controlled internal drug release (**CIDR**) removal and estradiol cypionate (**ECP**) treatment]. In suckled *Bos indicus* beef cattle with a CL 4 d before TAI, Meneghetti et al. (2009) reported an increase in P/AI (50.3 vs. 36.1%) when the $PGF_{2\alpha}$ was given 4 d before TAI rather than 2 d before TAI (time of CIDR removal and ECP treatment) of the protocol. In nonlactating *Bos indicus* beef cattle (Peres et al., 2009), treatment with $PGF_{2\alpha}$ 4 d before TAI resulted in lower P4 concentrations on the day of CIDR removal, a larger follicle at TAI, an increased percentage of cows that ovulated to the protocol (85.4% vs. 77.0%), and increased P/AI (52.0 vs. 36.4% for all cows; 60.9 vs. 47.2% for cows that ovulated) compared with cows treated with $PGF_{2\alpha}$ 2 d before TAI. In grazing dairy cows that were presynchronized with 2 $PGF_{2\alpha}$ treatments, increasing the interval from $PGF_{2\alpha}$ until GnRH and TAI from 58 to 72 h increased P/AI in a 5 d CoSynch TAI protocol (Ribeiro et al., 2012).

Recent studies from our laboratory evaluated the interval from $PGF_{2\alpha}$ until TAI in lactating dairy cattle synchronized with an E2 and P4-TAI protocol (Pereira et al., 2013a). Consistent with the results of previous studies using beef cattle, an increased interval between $PGF_{2\alpha}$ and TAI (2 vs. 3 d) increased P/AI in cows that received TAI (19.2 vs. 30.0% at d 60 pregnancy diagnosis), with a more subtle effect in cows that received timed embryo transfer (33.5 vs. 37.9%). The earlier $PGF_{2\alpha}$ treatment (3 d) resulted in a greater proportion of cows with low P4 concentrations (≤0.09 ng/mL) at TAI. Reduced P4 concentrations at TAI were associated with increased fertility, as has been observed in previous studies using GnRH-based TAI protocols (Souza et al., 2007; Martins et al., 2011; Giordano et al., 2012). In addition, reducing time from $PGF_{2\alpha}$ until induced ovulation decreased E2 concentrations near TAI, increased short luteal phases, and, at times, decreased P4 concentrations after AI (Vasconcelos et al., 2001; Bridges et al., 2010). Thus, the improved fertility with a longer time period from induction of luteolysis with $PGF_{2\alpha}$ until induction of synchronized ovulation could be related to greater time for CL regression and, thus, lower P4 at TAI or, alternatively, greater time for follicle growth, increased ovulatory follicle size, and increased E2 before synchronized ovulation.

The main hypothesis for the current study was that increasing the length of an estrous cycle synchronization protocol by 1 d would result in ovulation of a larger follicle and increased fertility in lactating dairy cows. The extra day of protocol length was added in the presence of lower P4 by advancing the time when the P4-releasing vaginal implant was removed after $PGF_{2\alpha}$ treatment. Thus, the experimental design allowed greater time for follicle growth in a lower P4 environment, as well as a longer time for complete CL regression before removal of the P4 implant. In addition to the main hypothesis, we also hypothesized that cows ovulating small follicles would have reduced fertility in either protocol. This study used the E2 and P4-based TAI program that is commonly used in Brazil and many other parts of the world.

MATERIALS AND METHODS

This study used only cycling cows in the cooler time of the year in Brazil to optimize the opportunity to observe any effects of protocol length or follicle size on fertility. This experiment was conducted at 3 commercial dairy farms in Minas Gerais, Brazil, from June to November 2011. All animal procedures followed the recommendations of the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 1999). During the experimental period, cows were housed in freestall barns with access to an adjoining sod-based paddock. Throughout the experiment, cows were milked 3 times daily. All procedures, including injections, ovarian ultrasonography, pregnancy diagnosis, blood collection, and TAI, were performed while cows were restrained in self-locking head gates at the feedline. Cows were fed a TMR based on corn silage and Tifton hay as forages ad libitum with a corn-soybean meal-based concentrate and minerals and vitamins, which were balanced to meet or exceed the nutritional requirements of lactating dairy cows (NRC, 2001).

Animals and Treatments

This study used a total of 759 lactating Holstein cows that were synchronized with an E2 or P4-based TAI program that is commonly used in Brazil and many other parts of the world. At the beginning of the experiment (d −11), cows averaged 153 ± 3.8 DIM, yielding 31.0 ± 0.30 kg of milk/d, with a BCS of 2.84 ± 0.02 [1 (emaciated) to 5 (obese) scale; Wildman et al., 1982], a lactation number of 1.82 ± 0.05 [primiparous (1), n = 473; multiparous (≥2), n = 286], and had been bred 2.16 ± 0.09 times. Within each farm, cows were blocked by parity (primiparous and multiparous); all cows that were past the voluntary waiting period and not pregnant were used and randomized into the study, without regard to whether they had been previously used in the study. Within each block, 1,101 cows were scanned to