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Comparison of 2 protocols to increase circulating progesterone concentration before timed artificial insemination in lactating dairy cows with or without elevated body temperature

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ABSTRACT

Two treatments designed to increase circulating progesterone concentration (P4) during preovulatory follicle development were compared. One treatment used 2 intravaginal P4 implants (controlled internal drug-releasing inserts; CIDR) and the other used a GnRH treatment at beginning of the protocol. Lactating Holstein cows that had been diagnosed as nonpregnant were randomly assigned to receive timed artificial insemination (TAI) following 1 of 2 treatments (n = 1,638 breedings): (1) GnRH: CIDR+ 2 mg of estradiol (E2) benzoate + 100 μg of GnRH on d −11, $PGF_{2\alpha}$ on d −4, CIDR withdrawal + 1.0 mg of E2-cypionate + $PGF_{2\alpha}$) on d −2, and TAI on d 0; or (2) 2CIDR: 2 CIDR + 2 mg of E2-benzoate on d −11, 1 CIDR withdrawn + $PGF_{2\alpha}$ on d −4, second CIDR withdrawn + 1.0 mg of E2-cypionate + $PGF_{2\alpha}$ on d −2, and TAI on d 0. Milk yield was measured daily between d 0 and d 7. Rectal temperature was measured using a digital thermometer at d 0 and 7, and elevated body temperature was defined as an average rectal temperature ≥39.1°C. Pregnancy diagnoses were performed on d 32 and 60 after TAI. We detected no effect of treatments on pregnancy per AI or pregnancy loss regardless of elevated body temperature, body condition score, parity, milk yield, or presence or absence of a corpus luteum (CL) on d −11 or d −4. Pregnancy per AI at 60 d was reduced [elevated body temperature = 22.8% (162/709), no elevated body temperature 34.1% (279/817)] and pregnancy loss tended to increase [elevated body temperature = 20.2% (41/203), no elevated body temperature 14.4% (47/326)] in cows with elevated body temperature. Various physiological measurements associated with greater fertility were also reduced in cows with elevated body temperature, such as percentage of cows with a CL at $PGF_{2\alpha}$ (decreased 7.9%), ovulatory follicle diameter (decreased 0.51 mm), expression of estrus (decreased 5.1%), and ovulation near TAI (decreased 2.8%) compared with cows without elevated body temperature. A greater proportion of cows (30.2%) had a CL at $PGF_{2\alpha}$ in the GnRH treatment [74.1% (570/763)] than in the 2CIDR treatment [56.9% (434/763)]; however, circulating P4 concentration was greater at the time of $PGF_{2\alpha}$ treatment (d −4) for cows 2CIDR (4.26 ± 0.13 ng/mL) than in cows in GnRH (3.99 ± 0.14 ng/mL). Thus, these 2 protocols yield similar fertility results that might be due to somewhat different physiological alterations. Treatment with GnRH increased the proportion of cows with a CL at $PGF_{2\alpha}$; however, the 2CIDR protocol increased circulating P4 under all circumstances.
Key words: synchronization protocols, progesterone, elevated body temperature

INTRODUCTION

Elevated progesterone (**P4**) during preovulatory follicle development is associated with greater fertility in cows submitted to AI after natural estrus (Folman et al., 1973; Erb et al., 1976; Fonseca et al., 1983; Meisterling and Dailey, 1987), after $PGF_{2\alpha}$-induced luteolysis (Diskin et al., 2006) or during timed AI (**TAI**) protocols (Giordano et al., 2010; Martins et al., 2011). Some of the reasons for reduced circulating P4 concentrations during growth of the preovulatory follicle in TAI protocols can be lack of a corpus luteum (**CL**) at the beginning of a synchronization protocol for first AI (Stevenson et al., 2008; Bisinotto et al., 2010a, 2013) or subsequent AI services (Fricke et al., 2003; Sterry et al., 2006; Silva et al., 2009), elevated steroid metabolism in lactating dairy cows (Sangsritavong et al., 2002; Vasconcelos et al., 2003; Wiltbank et al., 2006), or regression of the CL during the protocol (Thatcher et al., 1986; Araujo et al., 2009; Monteiro et al., 2015). Thus, lactating dairy cows can have reduced circulating P4 concentrations during preovulatory follicular devel-

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opment during natural or synchronized cycles, resulting in reduced pregnancy per AI (**P/AI**; Souza et al., 2008; Bisinotto et al., 2010a,b, 2013; Denicol et al., 2012; Dirandeh et al., 2015a,b; Wiltbank et al., 2015).

Two different approaches have been used to increase circulating P4 during preovulatory follicle development. First, treatment with GnRH at the beginning of a protocol can ovulate a follicle, even in the presence of a primary CL, thereby increasing circulating P4 by creation of an accessory CL. As expected, in GnRH-based protocols, increased circulating P4 concentration at the beginning of the protocol (Bisinotto et al., 2010b, 2013; Denicol et al., 2012) and the presence of a functional CL at the time of $PGF_{2\alpha}$ treatment have been associated with improved fertility (Giordano et al., 2010; Martins et al., 2011). Protocols that use GnRH on d 6 or 7 of the estrous cycle have a particularly high percentage of cows that ovulate to the GnRH treatment compared with cows treated with GnRH on other days of the cycle. This results in a new accessory CL, increased circulating P4, and better synchronization of the preovulatory follicular wave, resulting in increased P/AI with these protocols (Vasconcelos et al., 1999; Souza et al., 2008; Herlihy et al., 2012; Dirandeh, 2014; Wiltbank and Pursley, 2014). Similarly, addition of GnRH at the beginning of estradiol (**E2**)- and P4-based protocols results in greater circulating P4 at $PGF_{2\alpha}$ and greater fertility with these protocols (Pereira et al., 2015). Increased P4 during E2- or P4-based protocols is particularly important because these protocols result in lower circulating P4 concentrations at the time of $PGF_{2\alpha}$ treatment (2.29 ± 0.15 ng/mL) compared with GnRH protocols (2.89 ± 0.15 ng/mL; Vasconcelos et al., 2011a). In addition, ovulation to GnRH at the beginning of a synchronization protocol initiates a new follicular wave and may therefore improve synchronization of the follicular wave and potentially improve fertility during a TAI protocol. A second method to increase circulating P4 during preovulatory follicle growth is by using a P4 implant such as a controlled internal drug-releasing insert (**CIDR**). However, lactating cows have greatly elevated metabolism of circulating P4 due to elevated liver blood flow and this results in a suboptimal increase in circulating P4 (<1 ng/mL) during treatment with a single P4 implant (Stevenson et al., 2006). Alternatively, Bisinotto et al. (2013) evaluated supplementation of cows with low circulating P4 at the beginning of a protocol with 2 CIDR in a GnRH-based 5-d TAI program (d −8 GnRH, d −3 and −2 $PGF_{2\alpha}$, d 0 GnRH and AI). Treatment with 2 CIDR increased circulating P4 to 2.65 ng/mL and restored fertility in lactating dairy cows lacking a CL at the initiation of the TAI program to a value that was similar to cows that began the protocol with a CL, compared with cows with low circulating P4 without CIDR. In a recent study from our group (Pereira et al., 2017), we evaluated supplementation with 2 instead of 1 CIDR in an E2-/P4-based protocol in cows with reduced circulating P4 concentration at the beginning of the protocol (<1.0 ng/mL). Treatment with 2 CIDR tended to increase P/AI compared with 1 CIDR in TAI (1 CIDR: 42.8% vs. 2 CIDR: 52.6%; n = 326) but not in timed embryo transfer (n = 445) programs. Thus, increased circulating P4 and improved P/AI have been shown for protocols that produce a new CL using GnRH treatment or directly increase circulating P4 by treating with 2 P4 implants instead of 1 P4 implant during the protocol.

The objective of this study was to compare these 2 strategies for increasing circulating P4 concentration during preovulatory follicular development in lactating dairy cows. We hypothesized that treatment with GnRH or treatment with 2 CIDR would produce similar circulating P4 concentrations during the protocol, similar percentage of cows ovulating near TAI, and similar P/AI and pregnancy losses in lactating dairy cows.

MATERIALS AND METHODS

This experiment was conducted at 4 commercial dairy farms in Minas Gerais State, Brazil, from May 2013 to January 2014. 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 and had 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 ad libitum a TMR based on corn silage and Tifton hay as forages, with concentrates composed of corn and soybean meal, and added minerals and vitamins, which was balanced to meet or exceed the nutritional requirements of lactating dairy cows (NRC, 2001).

Animals and Treatments

This study used 1,638 breedings in lactating Holstein cows (throughout the text, "n" refers to the number of breeding events that received a particular protocol). At the beginning of the experiment (d −11), cows averaged 187.8 ± 13.5 DIM, yielding 31.4 ± 2.23 kg of milk/d, BCS of 2.94 ± 0.03 [on a 1 (emaciated) to 5 (obese) scale; Wildman et al., 1982], lactation number of 2.43 ± 0.36 [primiparous (=1) n = 457; multiparous (≥2) n = 1,181], and had been bred 3.38 ± 0.08 times.