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Hormonal manipulations in the 5-day timed artificial insemination protocol to optimize estrous cycle synchrony and fertility in dairy heifers

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ABSTRACT

Objectives were to determine the effects of GnRH at the initiation of the 5-d timed artificial insemination (AI) program combined with 2 injections of $PGF_{2\alpha}$ on ovarian responses and pregnancy per AI (P/AI) in dairy heifers, and the role of progesterone concentrations on LH release and ovulation in response to GnRH. In study 1, heifers received a controlled internal drug release (CIDR) insert containing 1.38 g of progesterone on d 0, an injection of 25 mg of $PGF_{2\alpha}$ and CIDR removal on d 5, and an injection of 100 μg GnRH concurrently with AI on d 8. Heifers were assigned to receive no additional treatment (control; n = 559) or an injection of GnRH on d 0 and a second injection of $PGF_{2\alpha}$ on d 6 (G2P; n = 547). In study 2, all heifers were treated as described for the control in study 1, and were allocated to receive no additional treatment (control; n = 723), an injection of $PGF_{2\alpha}$ on d 6 (NG2P; n = 703), or an injection of GnRH on d 0 and an injection of $PGF_{2\alpha}$ on d 6 (G2P; n = 718). In study 3, heifers received a CIDR on d 7 after ovulation and were assigned randomly to a low-progesterone (LP; n = 6) treatment in which 2 injections of 25 mg of $PGF_{2\alpha}$ each were administered 12 h apart, on d 7 and 7.5 after ovulation, or to a high-progesterone (HP; n = 12) treatment in which no $PGF_{2\alpha}$ was administered. On d 8, heifers received 100 μg of GnRH and blood was sampled at every 15 min from −30 to 180 min relative to the GnRH for assessment of LH concentrations. Additionally, 94 heifers were assigned to LP or HP and ovulation in response to GnRH was evaluated. In study 1, P/AI was greater for G2P than for the control on d 32 (59.4 vs. 53.5%) and 60 after AI (56.6 vs. 51.3%). In study 2, administration of GnRH on d 0 increased the proportion of heifers with a new corpus luteum on d 5 (control = 21.9 vs. NG2P = 20.1 vs. G2P = 34.4%). Administration of a second $PGF_{2\alpha}$ increased the proportion of heifers with progesterone <0.5 ng/mL at AI (control = 83.1 vs. NG2P = 93.0 and G2P = 87.2%). Pregnancy per AI was greater for G2P than for control and NG2P on d 32 (control = 52.9 vs. NG2P = 55.0 vs. G2P = 61.7%) and 60 (control = 49.0 vs. NG2P = 51.6 vs. G2P = 59.1%). In study 3, HP attenuated LH release and reduced ovulation (19.0 vs. 48.4%) in response to GnRH compared with LP. Combining GnRH and 2 doses of $PGF_{2\alpha}$ in the 5-d timed AI protocol improved follicle turnover, luteolysis, and P/AI in heifers. Elevated concentrations of progesterone suppressed LH release and are linked with the low ovulatory response to the initial GnRH treatment of the protocol.
Key words: 5-d timed artificial insemination, dairy heifer, luteolysis, ovulation

INTRODUCTION

Reproductive efficiency in dairy heifers affects age at first calving, which has a major impact on rearing costs and subsequent productive life (Gabler et al., 2000; Ettema and Santos, 2004). Most dairy operations in the United States use AI after observed estrus to manage reproduction in heifers (NAHMS, 2009). Nevertheless, advances in protocols for synchronization of the estrous cycle have supported the use of timed AI as an alternative method to manipulate reproductive cycles and improve economics when detection of estrus is less than 70% (Ribeiro et al. 2012b). Recent studies have consistently reported pregnancy per AI (**P/AI**) ranging from 50 to 60% in dairy heifers subjected to the 5-d timed AI program (Rabaglino et al., 2010; Lima et al., 2011), which are comparable to those observed in heifers inseminated at detected estrus (Kuhn et al., 2006). Further optimization of such programs to either simplify or improve fertility will likely increase acceptance by dairy producers.

Ovulation in response to the initial GnRH injection in timed AI programs enhances synchrony of the estrous cycle, shortens follicle dominance, and improves embryo quality and P/AI (Vasconcelos et al., 1999; Chebel et al., 2006; Cerri et al., 2009a). Nevertheless,

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only 15 to 35% of heifers ovulate when treated with GnRH at random stages of the estrous cycle (Stevenson et al., 2008; Lima et al., 2011). In addition, heifers that ovulate in response to the initial GnRH will have a newly formed corpus luteum (**CL**), which is generally refractory to a single treatment with $PGF_{2\alpha}$ on d 5 of the cycle (Rowson et al., 1972; Henricks et al., 1974). Eliminating the first GnRH reduced ovulation at the beginning of the synchronization protocol, but increased the proportion of heifers that underwent luteolysis at AI when a single $PGF_{2\alpha}$ injection was used (Lima et al. 2011). Because the benefits associated with follicle turnover were offset by a less-effective CL regression, P/AI did not differ between heifers that received or did not receive GnRH at the initiation of the timed AI program (Lima et al. 2011). These results indicate that the initial GnRH is not necessary when a single $PGF_{2\alpha}$ is used, which simplifies and reduce costs associated with the synchronization protocol.

Results from lactating dairy cows subjected to the 5-d timed AI program indicate that the use of 2 injections of $PGF_{2\alpha}$ administered 24 h apart improved CL regression and P/AI (Santos et al., 2010), particularly when ovulation to initial GnRH was high (Ribeiro et al., 2012a). Shorter intervals between $PGF_{2\alpha}$ treatments, ranging from 7 to 8 h, have been shown to increase P/AI compared with a single injection in beef cows (Kasimanickam et al., 2009), although preliminary results in dairy heifers did not confirm such benefit (Rabaglino et al., 2010). Therefore, it is reasonable to speculate that the combination of the initial GnRH and the administration of $PGF_{2\alpha}$ on d 5 and 6 of the protocol will improve follicle turnover and luteal regression, which are expected to increase P/AI.

The differences in catabolism of steroid hormones (Sangsritavong et al., 2002) explain the almost 1.5 ng/mL greater progesterone concentration in heifers than lactating cows during mid-diestrus (Sartori et al., 2004). In fact, the increase in progesterone concentrations with a controlled internal drug release (**CIDR**) insert is expected to be greater in nonlactating (Zuluaga and Williams, 2008) than in lactating cows (Cerri et al., 2009b). Progesterone affects LH secretion, which might compromise ovulatory response to GnRH treatment, which might partially explain the low ovulatory response to GnRH in dairy heifers. Results from beef heifers support this idea (Colazo et al., 2008; Dias et al., 2010).

It was hypothesized that a combination of GnRH at the initiation of the 5-d timed AI and injections of $PGF_{2\alpha}$ on d 5 and 6 of the protocol improves the synchrony of the estrous cycle and fertility in dairy heifers. Furthermore, it was hypothesized that elevated concentrations of progesterone compromise the release of LH and ovulation in response to a GnRH injection in dairy heifers. Study 1 was designed to compare a simplified 5-d timed AI protocol with a protocol that is expected to optimize P/AI by inducing ovulation and optimizing regression of newly formed CL. Study 2 was designed to evaluate the effects of GnRH at the initiation of 5-d timed AI program combined with 2 injections of $PGF_{2\alpha}$ on ovarian responses and fertility. Finally, the objectives of study 3 were to assess LH release and ovulation in response to GnRH in dairy heifers with low or high concentrations of progesterone in plasma.

MATERIALS AND METHODS

The University of Florida Institute of Food and Agricultural Sciences Animal Research Committee (Gainesville) approved all procedures in the 3 studies reported.

Study 1

Heifers, Diets, and Housing. A total of 1,106 nulliparous Holstein and crossbred Holstein-Jersey heifers at an average (±SD) of 14.0 ± 2.1 mo of age from 2 farms in north central Florida were enrolled in the study between March and June 2010. Crossbred heifers (n = 231) were located only in farm 2. Four hundred and fifty-seven heifers received their first insemination, whereas the remaining 649 heifers were diagnosed nonpregnant on d 32 after insemination and resynchronized to receive their second AI. Heifers in both locations were managed on pasture with access to portable shades and trees. Heifers were fed a TMR once daily formulated to meet or exceed the nutritional requirements of Holstein heifers weighing 360 kg and gaining 0.8 kg/d (NRC, 2001). The diet consisted of a mixture of lactating cow ration orts, Bermuda grass silage, wet brewers grain, and a mineral and vitamin supplement. For administration of hormonal treatments, insemination, and pregnancy examination, heifers were moved to an open-sided barn with self-locking stations in farm 1 or to a palpation rail in farm 2.

Experimental Design and Treatments. All heifers received a CIDR insert containing 1.38 g of progesterone (Eazi-Breed CIDR Cattle Insert; Zoetis Inc., Madison, NJ) on d 0, an i.m. injection of 25 mg of $PGF_{2\alpha}$ (dinoprost tromethamine; Lutalyse sterile solution; Zoetis Inc.) and CIDR removal on d 5, and an i.m. injection of 100 μg of GnRH (gonadorelin hydrochloride; Factrel; Zoetis Inc.) concurrently with AI on d 8. On d 0, heifers were blocked according to number of AI (first or second) and then age and, within each block, they were allocated randomly to receive no additional treatment (control; n = 559) or an injection of GnRH on d 0 and a second injection of $PGF_{2\alpha}$ on d 6 (**G2P**, n =