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# Treatment of lactating dairy cows with gonadotropin-releasing hormone before first insemination during summer heat stress

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#### ABSTRACT

The objectives of the experiments were to compare ovarian responses, pregnancy per artificial insemination, and pattern of insemination of 2 estrus detectionbased presynchronization protocols before first artificial insemination (AI) during heat stress. In experiment 1, primiparous lactating dairy cows (n = 1,358) from 3 dairies were assigned randomly to 2 treatments at 60  $\pm$  $3 (\pm SD)$  DIM (study d 0): (1) treatment with 100 µg of GnRH on study d 0 (Gpresynch), or (2) no treatment on study d 0 (control). In experiment 2, multiparous lactating dairy cows (n = 1.971) from 3 dairies were assigned randomly to 2 treatments at 49  $\pm$  3 ( $\pm$ SD) DIM (study d 0), similar to experiment 1. In both experiments,  $PGF_{2\alpha}$  injections were administered 14 d apart starting on study d 7 for all cows. Cows not inseminated after detection of estrus were submitted to a timed artificial insemination protocol at study d 35. In a subgroup of cows from 2 dairies, concentrations of progesterone were determined from blood samples collected on study d 0 and 7. Furthermore, ovaries were examined by ultrasonography on study d - 14, 0, and 7 to determine cyclic status and ovulation in response to GnRH treatment. In experiment 1, progesterone concentration was not different on d 0, but progesterone was increased for Gpresynch compared with control cows on study d 7 (3.6  $\pm$  0.3 vs. 2.7  $\pm$  0.4 ng/mL), respectively. Ovulation risk from study d 0 to 7 was increased for Gpresynch compared with control (50.6 vs. 15.2%). Control cows were inseminated at a faster rate than Gpresynch cows [adjusted hazard ratio (AHR) = 0.89, 95% confidence interval = 0.80 to 1.00, and the interaction between treatment and dairy affected pregnancy per artificial insemination at 36 and 94 d post-artificial insemination. In experiment 2, concentrations of progesterone did not differ on study d 0 or 7, despite ovulation risk from study d 0 to 7 being greater in Gpresynch than control cows (46.9 vs. 23.8%). The interaction between treatment and dairy affected hazard of insemination with Gpresynch cows from dairy 1 (AHR = 1.21; 1.05 to 1.41) being inseminated faster than control cows. Hazard of pregnancy was affected by treatment because Gpresynch cows became pregnant at a faster rate than control cows (AHR = 1.25; 1.04 to 1.50). In conclusion, GnRH-based presynchronization protocols initiated before the end of the voluntary waiting period may have benefits in reproductive efficiency of estrus detection-based programs during heat stress. In addition, treatment with GnRH decreased the prevalence of anovular cows at the initiation of PGF<sub>2α</sub> injections.

**Key words:** dairy cow, estrus detection, gonadotropinreleasing hormone, presynchronization

#### INTRODUCTION

Reproductive efficiency in dairy cattle has steadily declined during the past 45 yr (Lucy, 2001), with declines in reproductive performance greatest during summer because of heat stress (De Rensis and Scaramuzzi, 2003). Poor oocyte and embryo quality, decreased fertilization rates (Sartori et al., 2002), and increased embryonic and fetal mortality (Santos et al., 2004) partly explain the decreased fertility in cows exposed to heat stress during summer. The direct effect of heat stress on the gonads is one mechanism by which heat stress affects fertility. Heat-stressed dairy cows have altered hormone production and metabolism, which induce changes in the follicular micro-environment (Leroy et al., 2011).

The progesterone environment in which a follicle develops is believed to affect the quality of oocyte, and consequently, embryo quality (Wiltbank et al., 2014). Rivera et al. (2011) demonstrated that super-stimulated cows that had greater progesterone concentration before AI had a greater percentage of excellent/good and fair embryos recovered 6.5 d after AI, compared with cows with a lesser concentration of progesterone. Furthermore, increased pregnancy per AI ( $\mathbf{P}/\mathbf{AI}$ ) was

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detected in cows with follicles growing under greater concentration of progesterone before AI (Denicol et al., 2012). Although greater concentration of progesterone before AI has been studied in cows submitted to timed AI (**TAI**) protocols, little is known about greater concentration of progesterone in cows inseminated based on estrus (no TAI). Furthermore, others (Rivera et al., 2011; Denicol et al., 2012) did not investigate whether greater concentration of progesterone during dominant follicle selection had an effect during periods of summer heat stress, a time in which improving oocyte and embryo quality would be desired.

Several studies demonstrated that  $GnRH-PGF_{2\alpha}$ based presynchronization protocols for first TAI increased P/AI compared with only using  $PGF_{2\alpha}$  in lactating dairy cows (Stevenson et al., 2012; Ayres et al., 2013; Dirandeh et al., 2015). Cows exposed to presynchronization with  $PGF_{2\alpha}$  followed in 3 d by GnRH (**PG-3-G**), 7 d before Ovsynch, had greater P/AI compared with  $PGF_{2\alpha}$ -based presynchronization (Stevenson et al., 2012). The percentage of cows bearing a corpus luteum (CL) and progesterone concentration  $\geq 1$  ng/ mL was greater in the PG-3-G cows than the  $PGF_{2\alpha}$ based presynchronized controls at the time of the first GnRH injection of the TAI protocol (Stevenson and Pulley, 2012), indicating improved synchrony of the estrous cycle. Improved fertility of cows synchronized with a GnRH-PGF $_{2\alpha}$ -based presynchronization protocol compared with a  $PGF_{2\alpha}$ -based presynchronization protocol was partly related to the increased concentration of progesterone during dominant follicle selection (Avres et al., 2013).

A study conducted during summer demonstrated that synchronizing the first TAI with Double-Ovsynch, a  $GnRH-PGF_{2\alpha}$ -based presynchronization protocol, improved fertility in dairy cows compared with the Presynch-Ovsynch,  $PGF_{2\alpha}$ -based presynchronization protocol (Dirandeh et al., 2015). Although concentrations of progesterone were not evaluated, a greater proportion of Double-Ovsynch cows had a CL present at the first GnRH of the TAI protocol, which may have resulted in greater concentration of progesterone during dominant follicle selection before AI (Dirandeh et al., 2015). The reported improved fertility of cows submitted to PG-3-G (Stevenson and Pulley, 2012) and Double-Ovsynch (Dirandeh et al., 2015) during summer may be a result of increased concentration of progesterone as previously suggested (Avres et al., 2013). In contrast, the improved fertility may be consequence of increased fertility of anovular cows. The additional and multiple injections of GnRH in the PG-3-G and Double-Ovsynch protocols, respectively, are likely to induce ovulation during the protocol, which is believed to be beneficial for anovular cows. Because anovulation before first AI is associated with poor fertility (Bisinotto et al., 2010), inducing ovulation with an injection of GnRH during presynchronization protocols may have an additional benefit to anovular cows compared with protocols based only on  $PGF_{2\alpha}$ . Despite the potential benefits of using GnRH in presynchronization protocols, herds that strive to inseminate cows based on estrus detection may choose to use  $PGF_{2\alpha}$ -based presynchronization protocols in lieu of  $GnRH-PGF_{2\alpha}$ -based protocols because GnRH suppresses signs of estrus (Mendonça et al., 2012; Bruno et al., 2013; Chebel et al., 2013). Thus, a presynchronization protocol that induces estrus expression and ovulation of anovular cows, and increases concentration of progesterone during dominant follicle selection, may improve reproductive efficiency during summer heat stress in herds that submit cows to AI largely based on estrus detection.

We hypothesized that cows treated with GnRH before an estrus detection-based presynchronization protocol would induce ovarian cyclicity in anovular cows, increase concentration of progesterone, and improve P/ AI of heat-stressed cows. The objectives of the experiments were to compare ovarian response, P/AI, and pattern of insemination of 2 estrus detection-based presynchronization protocols for first insemination during summer heat stress.

#### MATERIALS AND METHODS

#### Cows, Housing, and Feeding

These experiments were conducted in 3 commercial dairy herds. Dairy 1 was located in the panhandle of Oklahoma, and dairies 2 and 3 were located in southwest Kansas. Lactating Holstein dairy cows in the 3 herds were enrolled in the experiments from June 2014 through September 2014. Cows from dairy 1 were housed in dry-lot corrals without shade and were milked thrice daily with a projected 305-d milk yield of 8,400 kg. Cows from dairy 2 were housed in dry-lot corrals with shade and were milked twice daily with a projected 305-d milk yield of 8,904 kg. Cows from dairy 3 were housed in free-stall barns equipped with fans and sprinklers, with access to a dirt exercise lot, and milked thrice daily with a projected 305-d milk yield of 9,017 kg. The 3 herds were fed a TMR once daily with ad libitum access to feed and water. Milk yields were recorded at each milking using a parlor management software program (DairyPlan C21, GEA Farm Technologies, Naperville, IL). Average daily milk yield during the week before enrollment was extracted from the on-farm software.

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