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Characterization of the variability and repeatability of gonadotropin-releasing hormone–induced luteinizing hormone responses in dairy cows within a synchronized ovulation protocol

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ABSTRACT

The primary objective was to determine the variability and repeatability of GnRH-induced LH responses. The secondary objective was to evaluate the associations among plasma LH, FSH, estradiol (E2), and progesterone (P4) concentrations. One hundred lactating Holstein cows (35 primiparous, 65 multiparous) were initially subjected to a presynchronization protocol (d 0, $PGF_{2\alpha}$; d 3, GnRH) followed 7 d later by Ovsynch (d 10, GnRH; d 17, $PGF_{2\alpha}$; 56 h later, GnRH) and timed artificial insemination 16 h after the last GnRH. Blood samples were collected immediately before the GnRH injection of presynchronization and the second GnRH of Ovsynch to determine plasma concentrations of LH, FSH, and P4. A second blood sample was collected 2 h after each of the above GnRH injections to determine GnRH-induced LH and FSH concentrations. Plasma concentrations of E2 were also determined in samples collected immediately before the second GnRH of Ovsynch. Cows that (1) had higher LH concentrations at 0 h than at 2 h after GnRH, (2) showed an ongoing spontaneous LH surge, (3) did not respond to GnRH, and (4) had P4 $\geq$ 0.5 ng/mL at GnRH of presynchronization and the second GnRH of Ovsynch were excluded from the analysis. The variability (coefficient of variation) and repeatability [between animal variance/(within animal variance + between animal variance)] of GnRH-induced LH response were determined from samples collected 2 h after the GnRH of presynchronization and the second GnRH of Ovsynch. The associations among plasma LH, FSH, E2, and P4 were determined at the second GnRH of Ovsynch. Mean (±SEM) LH concentrations before GnRH were 0.5 ± 0.04 and 0.6 ± 0.03 ng/mL, whereas mean LH concentrations 2 h after GnRH were 9.8 ± 1.0 and 12.1 ± 0.8 ng/mL at GnRH of presynchronization and the second GnRH of Ovsynch, respectively. The variability of GnRH-induced LH was 76.1 and 52.1% at GnRH of presynchronization and the second GnRH of Ovsynch, respectively. The repeatability estimate for GnRH-induced LH concentration between GnRH of presynchronization and Ovsynch assessments was 0.10. Plasma concentrations of LH were positively associated with FSH and E2 (r = 0.61 and 0.30, respectively) and negatively associated with P4 (r = −0.46) at the second GnRH of Ovsynch. In summary, GnRH-induced LH responses were highly variable and unrepeatable, and LH concentrations were positively associated with FSH and E2 and negatively associated with P4.
Key words: luteinizing hormone, variability, repeatability, progesterone

INTRODUCTION

A functional hypothalamic-pituitary-gonadal axis is essential for regulation of reproduction in both male and female mammals (Land, 1973). Gonadotropin-releasing hormone is a decapeptide synthesized and released by GnRH neurons in the hypothalamus that induces the release of FSH and LH from the anterior pituitary gland through receptor-mediated mechanisms (Kaltenbach et al., 1974; Fink, 1988). Progesterone (**P4**) and estradiol (**E2**) regulate FSH and LH release through positive and negative feedback mechanisms that act on the hypothalamus, anterior pituitary, or both (Goodman and Karsch, 1980; Karsch, 1987; Nett et al., 2002). Whereas FSH is required for follicular wave emergence (Adams et al., 1992), LH is essential for dominant follicle growth (Ginther, 2000), oocyte maturation (Hyttel et al., 1989), ovulation, corpus luteum development, and synthesis of P4 (Tomac et al., 2011). These events are critical for establishment and maintenance of pregnancy in domestic animals (Spencer et al., 2004). Therefore, selecting cows with greater

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capacity for LH secretion under defined conditions could be a strategy to improve fertility in dairy cows.

A phenotype that has high variability, repeatability, and heritability would be an ideal candidate for genetic selection. The variability and repeatability of other novel fertility traits such as anti-Müllerian hormone and antral follicle count (Burns et al., 2005; Ireland et al., 2008; Gobikrushanth et al., 2017) and their association with fertility outcomes have been of recent interest to many researchers (Mossa et al., 2012; Ribeiro et al., 2014). However, the variability, repeatability, and association with fertility under minimal influence of P4 have not been examined for GnRH-induced LH responses in dairy cows. Previous studies in rams and ewes (Haley et al., 1989) and beef cows (Webb et al., 1977; Williams et al., 1982; Williams and Stanko 1996; Fajersson et al., 1999) reported that GnRH-induced LH responses were variable between animals based on simple observations. However, none of the above studies quantified the variability with statistical analysis or were specific to lactating dairy cows. Endogenous LH release is pulsatile, resulting in a low correlation between repeated measures from the same animal (Haley et al., 1989); furthermore, measuring endogenous LH surge in large populations is impractical, making it an undesirable candidate trait for genetic selection. However, the induced LH surge response after exogenous GnRH administration may be a more useful endocrine parameter for investigating variability and repeatability. Previous studies reported a poor and nonsignificant repeatability for GnRH-induced LH responses when ram and ewe lambs (Tyrrell et al., 1980; n = 15 for each sex) and beef cows (Fajersson et al., 1999; n = 18) were repeatedly challenged with exogenous GnRH treatments. However, small sample sizes and variable concentrations of P4 might have contributed to nonsignificant repeatability estimates. In addition, although high heritability (h^2 = 0.44) and associations between high LH concentrations and fecundity have been reported in ewes, the associations were inconsistent (Haley et al., 1989). Similar conceptual studies evaluating the association between GnRH-induced LH responses and fertility in lactating dairy cows are lacking.

Evaluating the variability and repeatability of GnRH-induced LH response and establishing its association with fertility may identify it as a fertility phenotype to be considered in future genomic selection in dairy cows. We hypothesized that cows have variable responses to GnRH injection even under low P4 environment and those responses are repeatable. Therefore, our primary objective was to determine the variability and repeatability of GnRH-induced LH responses. The secondary objective was to evaluate the associations among plasma LH, FSH, E2, and P4 concentrations. In addition, the associations among LH response categories, FSH, E2, P4, and reproductive outcomes [i.e., ovulatory response, pregnancies per AI (**P/AI**), pregnancy at 60 d after AI and pregnancy loss] were also examined.

MATERIALS AND METHODS

Animals and Housing

The study was conducted at the Dairy Research and Technology Centre of the University of Alberta between November 2014 and September 2016. All the experimental procedures were approved by the University of Alberta's Animal Care and Use Committee for Livestock, and animals were cared for in accordance with the requirements of Canadian Council on Animal Care (2009). One hundred lactating Holstein cows (35 primiparous, 65 multiparous) were initially enrolled in the study. Cows were individually fed a total mixed ration (primary ingredients were barley silage, alfalfa silage, alfalfa hay, and concentrates) and housed in tie-stalls and let out for approximately 2 h of exercise during weekdays. Diets were formulated according to NRC (2001) to meet the requirements of a 650-kg lactating cow producing 45 kg of milk/d, and cows had ad libitum access to water.

Reproductive Management and Blood Sampling

Cows that were on average 52 (SD = 4.0; range = 45 to 59) DIM were placed on a modified G6G protocol and subjected to timed AI (Figure 1). In brief, the presynchronization protocol consisted of $PGF_{2\alpha}$ (d 0; Estrumate, 500 μg, i.m; Merck Intervet Corp., Kirkland, QC, Canada) and GnRH (d 3; Fertiline; 100 μg of gonadorelin acetate, i.m.; Vetoquinol N. A. Inc. Lavaltrie QC, Canada) administered 3 d apart. The Ovsynch protocol was initiated 7 d after the GnRH injection of the presynchronization program and involved i.m. injections of GnRH (d 10), $PGF_{2\alpha}$ (d 17), and GnRH 56 h later, followed by timed AI 16 to 20 h later (mean DIM = 72).

Transrectal ultrasonography (Aloka 500, Aloka Co Ltd., Tokyo, Japan) using a 7.5-MHz linear array transducer was first conducted at the time of the second GnRH of Ovsynch (~71 DIM) to confirm the presence of one or more putative ovulatory follicles (≥10 mm in diameter). Ovulation was confirmed on 73 DIM by the absence of the follicles that had been detected at the previous ultrasound examination. Ovulatory response was defined as the proportion of cows that ovulated after the second GnRH of Ovsynch. Transrectal ultrasonography of uterine contents was performed 33 d after AI and visualization of a viable embryo confirmed the