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Impact of phase of the estrous cycle and season on LH surge profile and fertility in dairy cows treated with different GnRH analogs (gonadorelin vs. buserelin)

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ABSTRACT

Our aim was to assess the GnRH-induced LH surge profile in dairy cows receiving two GnRH products (gonadorelin vs buserelin) given at proestrus or diestrus phase and to investigate whether season could alter LH surge profile in dairy cows. In Experiment 1, dairy cows at 108.2 ± 2.3 DIM, producing 41.5 ± 0.3 kg/day were randomized to receive, during proestrus and diestrus: Ovarelin® i.m. (OVA; n = 56; 100 mg of gonadorelin diacetate tetrahydrate; Ceva Animal Health, France) or Receptal® i.m. (REC; n = 52; 10 mcg of buserelin diacetate; MSD, Germany). In Experiment 1, blood samples were collected at hour 0 (just before GnRH treatment) at 30min, 1 h and then hourly until 5 h post-GnRH. In Experiment 2, cows were synchronized with a modified G-6-G protocol and randomized to receive either OVA or REC throughout the synchronization program. In Experiment 1, peak LH concentrations (ng/mL) were not affected by type of GnRH (OVA = 6.2 ± 0.4 vs REC = 6.7 ± 0.4; P = 0.37) or season (Cool = 6.8 ± 0.4 vs Warm = 6.1 ± 0.4; P = 0.22), and there were no interactions between GnRH type and phase of the estrous cycle or season. Interestingly, the area under the curve (AUC) of LH release (ng/ml*time) was significantly lower during warmer months (Cool = 20.3 ± 1.2 vs Warm = 16.9 ± 1.1; P = 0.04). As expected, LH peak was affected by phase of the cycle (proestrus = 8.2 ± 0.4 vs diestrus = 4.7 ± 0.4; P < 0.01). Ovarelin caused LH concentrations to increase faster, reaching highest concentration sooner (h) than REC (1.5 ± 0.1 vs 2.3 ± 0.1; P < 0.01). As a result, cows receiving OVA had greater circulating LH concentrations (ng/mL) at 1 h after GnRH treatment than cows receiving REC (P < 0.01). In contrast, cows treated with REC had longer (P = 0.01) intervals from peak until return to nadir. In Experiment 2, pregnancy per AI (P/AI) was similar for cows receiving either GnRH product during the synchronization protocol, with no detectable interactions between GnRH type and season. In conclusion, phase of the estrous cycle had a great impact on the GnRH-induced LH surge profile and cows during warm season had reduced AUC. Additionally, type of GnRH did not influence LH surge profile and P/AI in synchronized cows. Regardless of GnRH type, strategies to avoid heat stress and excessively high levels of circulating P4 near the time of GnRH treatment might help improve LH release profile in dairy cows, and ultimately increase P/AI.

1. Introduction

Currently, several types of native-like GnRH products and synthetic GnRH analogs are commercially available for use in cattle. The potency of the native GnRH has been altered in synthetic analogs through amino acid modification mainly in positions 6 and 10 of the molecule [1]. Although potency may vary among commercially available GnRH products, the recommended label dose for each product is normally set at higher or lower doses depending on the potency of the analog in order to produce adequate LH release to induce ovulation of a dominant follicle [2].

A number of factors may alter LH surge response following a

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GnRH treatment including circulating progesterone levels [3] and heat-stress [4]. For example, Giordano et al. [3] clearly showed that greater circulating progesterone in lactating dairy cows can drastically reduce the GnRH-induced LH surge profile. Furthermore, latest reports have shown much greater synchronization rates and fertility in cows that ovulate to the GnRH treatment at the beginning of Ovsynch-like timed AI protocols as compared to those that did not ovulate [5,6]. This has major practical implications to farmers since GnRH analogs are commonly used in association to synchronization protocols for timed AI [7,8] in cows having different circulating concentrations of progesterone in blood when the synchronization protocol begins.

Heat stress is widely known to be a major detrimental factor impairing reproductive performance in dairy cows worldwide [9]. For example, heat stress has been described to disturb follicle growth and increase rate of double-ovulations [9]. Although poorly described in the literature, heat-stress may even act lowering LH peak in response to a GnRH treatment [4]. However, Gilad et al. [4] only looked at the effects of heat stress during low circulating progesterone at proestrus phase. De Rensis et al. [10] studied the effect of GnRH or hCG in synchronized cows both in the winter and summer, but their focus was not on the resulting LH profile during the two seasons. Thus, information of possible detrimental effects of warmer temperatures on LH surge during diestrus (high circulating P4) is currently lacking.

Therefore, the first objective of this study was to compare the GnRH-induced LH surge profile after treating cows with two commercially available GnRH products (gonadorelin vs. buserelin; Experiment 1), given both at a low (proestrus) and high (diestrus) circulating P4 environment in lactating dairy cows. The second objective was to assess the impact of heat-stress on the LH surge profile following the GnRH treatment (Experiment 1) and fertility in lactating cows synchronized for 1st postpartum AI with a GnRH-Prostaglandin (PGF2α) based protocol (Experiment 2). Our main hypothesis was that heat-stress would reduce the LH surge regardless of phase of the estrous cycle and type of GnRH analog.

2. Materials and methods

2.1. Animal handling, housing and experimental design

During experimental periods, all animals enrolled in Experiment 1 and 2 were handled and treated according to the Canadian Council on Animal Care (CCAC) guidelines for farm animals available at http://www.ccac.ca/en_/standards/guidelines. To keep animals comfortable in warmer months, all lactating cows were kept in shaded pens equipped with fans above cubicles as well as fans and sprinklers in feed line and milking parlor waiting area. Typical average temperature, humidity and THI index in which animals were exposed to at this region of Spain has been reported elsewhere [11]. Briefly, maximum THI index reaches 80° in warmer months and in cooler months is down to 50°. Cows had water *ad libitum* and were fed a TMR that was based on hay, corn silage, and alfalfa silage as forage supplemented with concentrates of corn and soybean meal. The TMR was balanced to meet or exceed minimum nutritional requirements for lactating dairy cows (NRC, 2001).

2.1.1. Experiment 1

Lactating dairy cows (n = 108) at 108.2 ± 2.3 DIM, producing 41.5 ± 0.3 kg/day from two commercial free-stall dairies in Northeastern Spain were randomized to receive, during warmer (July/August 2014) and cooler (September to November 2014) months and within each season cows were at proestrus (at last GnRH of the modified G-6-G synchronization protocol [6] represented in Fig. 1) or diestrus (at 7 days after synchronized ovulation), one of the two

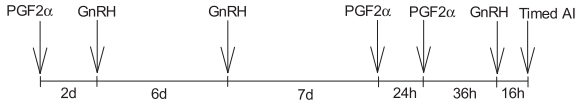

Fig. 1. Modified G-6-G protocol [6] to synchronize high producing dairy cows for 1st postpartum AI (Experiment 2). PGF2α: Enzaprost® (Dinoprost Tromethamine, Ceva Animal Health). GnRH: gonadorelin (Ovarelin®) or buserelin (Receptal®).

GnRH analogs: Ovarelin® (OVA; n = 56; 100 mg of gonadorelin diacetate tetrahydrate, CEVA Animal Health, France) or Receptal® (REC; n = 52; 10 mcg of buserelin diacetate, MSD, Germany).

Blood samples were collected from the caudal vein into 10 ml heparinized vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) just before the GnRH treatment to measure circulating levels of progesterone (P4) and also at 0 h, 30min, 1 h, 2 h, 3 h, 4 h and 5 h after GnRH to measure circulating LH concentration. All samples were immediately centrifuged (3000×*g* for 20 min), and serum was harvested and stored at −20 °C until the assay was performed. Only cows having circulating P4 < 1.0 ng/ml at proestrus and >1.0 ng/ml at diestrus were considered in the final data analysis.

2.1.2. Experiment 2

Lactating dairy cows (n = 685) from the same commercial herds in Spain were 61–67 days in milk and randomized from May 2014 to June 2015 based on even/odd ear tag numbers to receive OVA or REC throughout a G-6-G synchronization protocol [6] for 1st postpartum artificial insemination (AI). Trans-rectal ultrasonography (Easi Scan, 4.5 MHz - 8.5 MHz, BCF Technology Ltd., Scotland) was used to perform pregnancy diagnosis at 32 ± 3 days post AI and to confirm the pregnancy status at 62 ± 3 days post AI. An additional ultrasonography exam was performed 21 days after calving to detect possible uterine diseases as previously described [12]. Briefly, cows classified as having postpartum diseases were animals diagnosed with retained fetal membranes, acute puerperal metritis, clinical metritis, endometritis, pyometra, ketosis, acute clinical mastitis, left displaced abomasum, lameness, pneumonia and ovarian disorders such as ovarian cysts according to [12,13]. Production, health, fertility and management-related data were recorded by the herd manager and the attending veterinarians using a specific farm software (Afimilk, Kibbutz Afikim, 1514800, Israel).

2.2. Hormone assays

Circulating P4 for all samples was analyzed in a single assay in single sample aliquots performed using a solid-phase radioimmunoassay (Coat-a-Count Progesterone, Diagnostic Products Corporation, Los Angeles, CA) as previously described [14]. The intra assay coefficient of variation in the P4 assay was 4.6%. LH concentrations were determined with a validated homologous double-antibody radioimmunoassay (RIA) in duplicate aliquots of 100 μL of plasma, according to Dieleman et al. [15]. The LH inter-assay coefficient of variation was 2.3%. Intra assay coefficient of variation for the LH assay was 9.6%. Specificity for the LH assay was 0.39 ng/ml and for the P4 assay was 0.1 ng/ml and were defined as ½ the value of the lowest concentration point in the standard curves.

2.3. Statistical analysis

The repeated-measures analysis was performed using the procedure MIXED of SAS [16] version 9.3, SAS Institute Inc., Cary, NC. The model included the effects of treatment, season, stage of the estrous cycle, sampling time, the interaction between treatment and time, and cow, which was treated as a random effect and was