J. Dairy Sci. 100:1–12
https://doi.org/10.3168/jds.2016-12513


# Intravaginal instillation of gonadotropin-releasing hormone analogues with an absorption enhancer induced a surge of luteinizing hormone in lactating dairy cows

**R. Wijma, M. L. Stangaferro, M. Masello, M. A. Elmetwally,[1] G. E. Granados, F. Amovilli, and J. O. Giordano[2]**
Department of Animal Science, Cornell University, Ithaca, NY 14853

## ABSTRACT

Our objectives were to evaluate circulating LH concentrations after intravaginal (IVG) instillation of GnRH analogs in lactating dairy cows. In 2 experiments, lactating Holstein cows (experiment 1: n = 32; experiment 2: n = 47) received the experimental treatments 48 h after the first of 2 $PGF_{2\alpha}$ treatments given 12 h apart and 7 d after a modified Ovsynch protocol (GnRH at −7 d, $PGF_{2\alpha}$ at −24 h, $PGF_{2\alpha}$ at −56 h, GnRH at 0 h). In experiment 1, cows were stratified by parity and randomly allocated to receive the following treatments: 2 mL of saline IVG (SAL, n = 6), 100 μg of gonadorelin (Gon) i.m. (G100-IM, n = 5), and 100 (G100, n = 7), 500 (G500, n = 8), or 1,000 μg of Gon IVG (G1000, n = 7). In experiment 2, treatments were SAL (n = 8), G100-IM (n = 8), G1000 (n = 7), 1,000 μg of Gon plus 10% citric acid (CA) IVG (G1000CA, n = 8), 80 μg of buserelin IVG (B80, n = 8), and 80 μg of buserelin plus 10% CA IVG (B80CA, n = 8). In both experiments, blood was collected every 15 min from −15 min to 4 h, and every 30 min from 4 to 6 h after treatment. Data for area under the curve (AUC), mean LH concentrations, and time to maximum LH concentration were analyzed by ANOVA with (mean LH only) or without repeated measures using PROC MIXED of SAS (version 9.4, SAS Institute Inc., Cary, NC). The proportion of cows with a surge of LH was evaluated with Fisher's exact test using PROC FREQ of SAS. In both experiments, LH concentrations were affected by treatment, time, and the treatment by time interaction. In experiment 1, the AUC for LH and maximum LH concentration were greatest for the G100-IM treatment and were greater for the G1000 than for the SAL and G500 treatments. The proportion of cows with an observed surge of LH was 100 and 0% for cows that received Gon i.m. and IVG, respectively. In experiment 2, the AUC and maximum LH concentrations were greater for the G100-IM, G1000CA, and B80CA treatments than for the other IVG treatments. The proportion of cows with a surge of LH differed by treatment (SAL = 0%, G100-IM = 100%, G1000 = 14%, G1000CA = 88%, B80 = 13%, and B80CA = 100%). For the treatments with a surge of LH, time to maximum concentration of LH was the shortest for the G100-IM treatment, intermediate for the G1000CA treatment, and the longest for cows in the B80CA treatment. In conclusion, Gon (up to 1,000 μg) absorption through intact vaginal epithelium after a single IVG instillation was insufficient to elicit a surge of LH of normal magnitude. Conversely, IVG instillation of 1,000 μg of Gon and 80 μg of buserelin with the addition of citric acid as absorption enhancer resulted in a surge of LH of similar characteristics than that induced after i.m. injection of 100 μg of Gon.

**Key words:** intravaginal, gonadotropin-releasing hormone analogue, citric acid, luteinizing hormone surge

## INTRODUCTION

Timed AI is one of the most widely used biotechnologies in cattle operations around the world (Lamb et al., 2010; Wiltbank and Pursley, 2014). In dairy farms, systematic implementation of synchronization of ovulation protocols ensures timely insemination (Pursley et al., 1997; Fricke et al., 2003) and improves fertility outcomes (Moreira et al., 2001; Souza et al., 2008; Giordano et al., 2012b). Synchronization of ovulation protocols are continuously evolving to optimize follicle development, luteal regression, timing of ovulation, and the endocrine environment before and after timed AI. As a result, groups of cows must receive hormonal treatments on multiple days of the week and different times of the day, which may reduce protocol compliance and success in farms without appropriate facilities, frequent access to animals, and availability of qualified labor. Frequent cow manipulation also disrupts time

Received December 24, 2016.
Accepted May 20, 2017.
[1]Current address: Faculty Veterinary Medicine, Mansoura University, Mansoura, Egypt.
[2]Corresponding author: jog25@cornell.edu

budgets and normal behavior. Thus, fully automated hormone delivery systems may be an alternative to individual injections for facilitating implementation and improving compliance with synchronization of ovulation protocols. Automating hormone delivery may also allow designing more effective treatments or treatments tailored to individual or subgroups of cows based on their physiological or health status.

Ideally, the body cavity to insert and hold an automated hormone delivery system should allow holding the device for prolonged periods of time and easy access to the device for removal after use. Because the vagina meets these criteria, fully automated electronically controlled intravaginal (**IVG**) drug delivery devices have been described (Rathbone et al., 1998; Cross et al., 2004; Künnemeyer et al., 2004) and could be developed for use in cattle. Another important attribute of a body cavity to insert an automated hormone delivery device is to allow proper absorption of hormones to elicit the desired physiological response. Although sustained release of progesterone (**P4**) through nonautomated delivery devices has been extensively studied and is currently used in synchronization of estrus and ovulation protocols (Macmillan et al., 1991; Macmillan and Peterson, 1993; Chebel et al., 2006), the feasibility of IVG administration of important reproductive hormones, such as GnRH and $PGF_{2\alpha}$, has rarely been studied and IVG administration is not currently used in cattle. Beyond potential differences in molecular structure that may affect IVG absorption, a fundamental difference between IVG treatments with P4 and hormones such as GnRH and $PGF_{2\alpha}$ is that the former can effectively exert its biological function through sustained release because an acute effect is not necessary. Conversely, administration of exogenous GnRH is only effective to induce a surge of LH and $PGF_{2\alpha}$ to trigger luteolysis through immediate absorption and an acute effect on their target tissues (i.e., pituitary gland for GnRH and corpus luteum for $PGF_{2\alpha}$).

The feasibility of inducing a surge of LH of similar magnitude after IVG than i.m. administration of GnRH or its analogs has been previously studied in other species, such as the sow (Stewart et al., 2010), the rat (Okada et al., 1982, 1983), and the rabbit (Viudes-de-Castro et al., 2007), but not in cattle. Although GnRH absorption through epithelial walls can occur through transmembrane diffusion, vesicle receptor-mediated transport, or paracellular diffusion (Richardson and Illum, 1992), vaginal absorption may be challenging. Transmembrane transport is probably limited due to the hydrophilic nature of GnRH, and a receptor-mediated transport mechanism in the vaginal epithelium seems unlikely. Further, paracellular transport may be limited by intercellular apical junction complexes (Hussain and Ahsan, 2005) formed by tight junctions and adherens junctions (Ivanov et al., 2005).

To overcome challenges associated with absorption efficiency of molecules through intact tissues, absorption enhancers can be included to disrupt the integrity of intercellular junctions so that paracellular transport increases (Okada et al., 1982, 1983; Fatakdawala and Uhland, 2011). For example, using a rat model, Okada et al. (1982, 1983) observed an increase in absorption and vaginal permeability to leuprolide (i.e., a GnRH analog) when including citric acid in the vehicle. Carboxylic acids, such as citric acid, chelate calcium, which has been shown to loosen intercellular tight junctions, thus facilitating intercellular transport of molecules (Cho et al., 1989). In addition to absorption enhancers, a potential strategy to increase the efficacy of hormones administered through the IVG route may be the use of more potent hormone analogs. Although absorption of all analogs may be equally compromised, more potent analogs could be more effective because smaller amounts are needed to elicit a satisfactory physiological response. In the case of GnRH, multiple analogs of varying potency are available. For example, buserelin has been shown to be up to 50 times more potent than gonadorelin (Chenault et al., 1990; Picard-Hagen et al., 2015), thereby buserelin may be an alternative to gonadorelin for IVG administration.

We performed 2 experiments to evaluate the feasibility of inducing a surge of LH after IVG instillation of GnRH analogs in lactating dairy cows. We aimed to determine if it was possible to induce a surge of LH of similar magnitude, timing, and duration after IVG instillation of GnRH analogs as after i.m. injection of the labeled dose of gonadorelin to induce ovulation in cattle (i.e., 100 μg). Specifically, the objective of experiment 1 was to compare circulating LH concentrations after IVG instillation of different doses of the GnRH analog gonadorelin. We hypothesized that IVG instillation of gonadorelin would induce a surge of LH similar to that observed after i.m. injection of 100 μg of gonadorelin. Also, we hypothesized that instillation of greater doses of gonadorelin would result in greater circulating LH concentrations. Based on the results of experiment 1, we conducted a second experiment to evaluate LH concentrations after IVG instillation of gonadorelin or buserelin with or without the inclusion of citric acid as an absorption enhancer. We hypothesized that the inclusion of citric acid to the GnRH solution and the use of a GnRH analog of greater potency than gonadorelin (i.e., buserelin) would result in a surge of LH similar to that observed after i.m. injection of 100 μg of gonadorelin.