Ovarian Characteristics, Serum Hormone Concentrations, and Fertility in Lactating Dairy Cows in Response to Equine Chorionic Gonadotropin

S. L. Pulley, L. D. Wallace, H. I. Mellieon, and J. S. Stevenson

Summary

The objective of this study was to evaluate the effects of equine chorionic gonadotropin (**eCG**) on various characteristics associated with an effective timed artificial insemination (**AI**) protocol in lactating dairy cows. Cows (n = 121) in a single herd were treated with 2 injections of prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**) 14 days apart (**Presynch**), with the second injection administered 11 days before the onset of a timed AI protocol. Cows received either saline or 400 IU eCG concurrent with the $PGF_{2\alpha}$ injection of the Ovsynch protocol (injection of gonadotropin-releasing hormone or GnRH, 7 days before and 48 to 56 hours after $PGF_{2\alpha}$ with insemination occurring 12 to 16 hours after the second GnRH injection). Blood samples were collected during the study to monitor serum changes in progesterone and estradiol in order to determine if eCG would facilitate increased estrual activity, improved ovulatory response, and enhanced postovulatory luteal function. Administration of eCG tended to increase the number of corpora lutea (**CL**) and on days 9 and 16 after $PGF_{2\alpha}$, corresponding to days 6 and 13 postovulation, but the volume of the luteal tissue was less than that in the control. Timed AI pregnancy rates did not differ between eCG (36.9%) and control cows (41.8%). We concluded that use of eCG provided no profertility advantages to dairy cattle when programmed for a timed insemination at first service.

Key words: corpus luteum, equine chorionic gonadotropin, follicle, progesterone, timed artificial insemination (AI)

Introduction

Cows inseminated at first service after detected estrus have better conception rates than those inseminated by appointment in an ovulation-synchronization program (40.7 vs. 33.5%; Stevenson and Phatak, Dairy Research 2009, Report of Progress 1021, pp. 56-62), but not as good as cows in estrus at the timed artificial insemination (**AI**; 51.8%). Earlier work demonstrated that when estradiol cypionate was administered as part of a timed AI program, pregnancy outcomes tended to be improved. Because dairy cows have greater rates of metabolism of steroids and produce large quantities of milk, their circulating concentrations of progesterone and estradiol are less in comparison with nonlactating heifers despite having larger corpora lutea (**CL**) and larger follicles, respectively. It seems that some lactating dairy cows lack sufficient serum concentrations of estradiol to support and facilitate strong estrual activity.

Equine chorionic gonadotropin (**eCG**) is a member of the glycoprotein family, which includes follicle stimulating hormone (FSH) and luteinizing hormone (LH). It is secreted by the trophoblast cells of the chorionic girdle in the pregnant mare beginning approximately at 37 days of gestation. In the mare, eCG elicits a similar response to that of LH. In contrast, in other species, eCG acts like FSH. The FSH properties of eCG have led to its use as an exogenous hormone preparation for stimulation of follicular growth and superovulation in farm animals.

Administration of eCG has been shown to increase the diameter of the preovulatory follicle, thus suggesting the potential for increasing circulating estradiol concentrations and more estrual activity. Despite a similar-sized preovulatory follicles and CL, administration of eCG increased circulating progesterone concentrations in cross-breed lactating beef cows and Holstein dairy cows. Equine CG may serve as an exogenous treatment to increase plasma concentrations of progesterone and estradiol, while stimulating estrual activity.

The objective of this study was to evaluate the effects of eCG administration on preovulatory follicle diameter, circulating estradiol and progesterone concentrations, CL diameter, estrous activity, and pregnancy rates in lactating dairy cows whose ovulation is synchronized before first service.

Experimental Procedures

Lactating Holstein cows (72 multiparous and 49 primiparous) from the Kansas State University Dairy Teaching and Research Center were enrolled in the study. Cows were housed in covered free stalls and fed twice daily a total mixed ration (TMR) calculated to meet or exceed the nutrient requirements for a lactating Holstein cow producing 110 lb of milk with 3.5% milk fat. The diet consisted of corn silage, sweet bran, cracked corn, alfalfa hay, whole cottonseed, soybean meal, vitamins, and minerals. Seventeen breeding clusters were formed every 14 days as cows and heifers calved. Cows were blocked by lactation number and days in milk, and assigned randomly to 2 treatments (Figure 1). Body condition scores (BCS; 1=thin and 5=fat) were assigned on day −7 for each cow. Inseminations occurred between April and November 2009. Cows were assigned to treatments at random stages of the estrous cycle and administered 2 $PGF_{2\alpha}$ injections (Presynch; 2 mL Estrumate, Schering-Plough Animal Health, Union, NJ; 5 mL Lutalyse, Pharmacia Animal Health, New York, NY) injections 14 days apart followed by a timed AI protocol (Ovsynch). The Ovsynch protocol consisted of 2 injections of 100 μg gonadotropin-releasing hormone (GnRH; Fertagyl, Intervet, Millsboro, DE) 9 days apart with a 25 mg $PGF_{2\alpha}$ injection administered 48 hours before a second injection of GnRH. Cows received either 400 IU eCG (Novormon 5000, Vetrepharm Canada Inc., London, ON; Pregnecol 6000, Bioniche, Belleville, ON) concurrent with $PGF_{2\alpha}$ or no further treatment (control). Cows were inseminated 72 hours after $PGF_{2\alpha}$ (16 hours after the second GnRH injection). Pregnancy was initially diagnosed at 33 days post-AI and reconfirmed at 61 days by transrectal ultrasonography. Pregnancy loss was calculated between the 2 pregnancy diagnoses.

All cows were fitted with HeatWatch transmitters (Cow Chips LLC, Denver, CO) on day −7 to quantify standing estrus and characteristics of estrus during the 96-hour period encompassing the breeding week (post–$PGF_{2\alpha}$ injection). Transmitter function was tested on day −7 and 0. Cows were moved into a dirt lot twice daily for 30 minutes during the breeding week for visual detection of estrus and to aid in expression of estrous activity. Cows were considered to be in standing estrus when 2 mounts of 2 seconds or longer in duration within a 24 hour period were recorded by the HeatWatch system.

Ovaries of all cows were examined via transrectal ultrasonography using an Aloka 500V ultrasound scanner equipped with 5.0 MHz linear probe to determine the structures present in each ovary on days −7, 0, 2, 4, 9, and 16. A structural map of each ovary was drawn with the position and diameter of follicles ≥5 mm in diameter and each CL, which allowed for evaluation of visual luteolysis and ovulatory response to both GnRH injections (days −7 and 4), preovulatory follicle diameter, and CL diameters on days 9 and 16. Follicle diameter was determined by av-

eraging the measurements of follicular width and length taken at the widest point and perpendicular to the first measurement using the internal calipers of the Aloka 500V. Ovulation on day 4 was defined as disappearance of one or more follicles ≥8 mm in diameter from an ovary, which a follicle had been recorded on the previous scan of that ovary, followed by the formation of a CL (Figure 1).

Blood was sampled from all cows by puncture of the coccygeal vein or artery into evacuated tubes (BD Vacutainer, Franklin Lakes, NJ). Blood samples were collected days −7, 0, 2, 4, 9, and 16 for progesterone assay. At pregnancy diagnosis, on days 33 and 61, blood samples also were collected from pregnant cows for later progesterone assay. Additional blood samples were collected every 24 hours from days 0 to 3 for estradiol assay. Samples were immediately cooled and stored at 5°C for 16 hours. Blood tubes were centrifuged at 1,000 x g for 15 minutes in a refrigerated centrifuge at 5°C for serum separation and harvest. Serum samples were frozen and stored at −20°C until assayed for progesterone and estradiol by radioimmunoassay.

Results and Discussion

Results are summarized by treatment in Table 1. Progesterone concentrations did not differ between treatments at $PGF_{2\alpha}$ injection on day 0 of the protocol or 24 hours before timed AI. Regression of the CL after $PGF_{2\alpha}$ injection on day 0 did not differ between treatments whether determined by visual observation of the CL (monitored by transrectal ultrasonography) or by changes in serum progesterone concentration. Likewise, estradiol concentrations did not differ between treatments at $PGF_{2\alpha}$ injection or 24 hours before timed AI. Incidences of single or multiple ovulations in response to the first or second GnRH injection did not differ between treatments. Volume of the CL on day 9 was not different between treatments. In contrast, the CL volume on day 16 was greater ($P = 0.04$) for cows receiving eCG treatment, but concentrations of progesterone did not differ between treatments. Cows treated with eCG tended ($P < 0.10$) to have an increased number of CL on day 9 and 16 (Table 1).

A treatment by time interaction was observed for estradiol concentrations during the collection period. Concentrations of estradiol gradually increased from (eCG = 2.45 vs. Control = 2.68 pg/mL) day 0 to day 2 (4.24 vs. 4.0 pg/mL), at which time concentrations progressively decreased (2.0 vs. 2.0 pg/mL). Estradiol concentrations did not differ between treatments from days 0 to 3 (Figure 2).

Concentrations of progesterone decreased rapidly from days 0 to 2 after the injection of $PGF_{2\alpha}$ administered on day 0. Increased progesterone concentrations from days 2 to 4 are the result of GnRH-induced ovulation of a preovulatory follicle and subsequent CL formation. Progesterone concentrations, however, did not differ between treatments from days 0 to 4 after eCG treatment.

Pregnancy rates did not differ between treatments at either 33 or 61 days (Table 2). Pregnancy loss between the first and second pregnancy diagnoses was numerically lesser for the eCG treated cows; however, no significant difference was detected between treatments.

Administration of eCG failed to change any characteristic studied except for increased luteal volume by day 16 postovulation. Increased luteal volume has been previously observed in beef cattle treated at similar times relative to AI. Despite increased luteal volume by day 16, we were unable to detect an increase in progesterone concentration as reported in beef cattle.

Table 1. Effect of equine gonadotropin (eCG) on steroid concentrations, ovarian structures, and ovulation characteristics in lactating dairy cows

Item	Treatment[1] eCG	Control	P-value
Progesterone, ng/mL			
At $PGF_{2\alpha}$	5.75 ± 0.3	5.69 ± 0.3	0.90
24 hours before AI	0.82 ± 0.3	0.43 ± 0.3	0.41
2 days post-AI	0.63 ± 0.3	0.79 ± 0.3	0.73
CL regression[2]			
Visual, %	97.0	94.0	0.51
Based on changes in progesterone, %	80.0	82.0	0.89
Estradiol, pg/mL			
At $PGF_{2\alpha}$	2.45 ± 0.2	2.84 ± 0.3	0.29
24 hours before AI	4.24 ± 0.2	4.00 ± 0.3	0.52
First GnRH			
Ovulation[3], %	63.6 ± 0.5	69.1 ± 0.5	0.52
Double ovulation[4], %	21.4 ± 0.4	19.0 ± 0.4	0.86
Second GnRH			
Ovulation[5], %	96.9	100.0	0.15
Double ovulation[6], %	20.3	18.2	0.73
Ovulatory follicle diameter, mm			
Primary follicle	13.9 ± 0.3	15.0 ± 0.48	0.08
Secondary follicle	11.0 ± 0.4	13.9 ± 0.5	0.004
Corpora lutea (CL) volume, cm³			
Day 9	6.57 ± 0.5	6.97 ± 0.51	0.40
Day 16	9.1 ± 0.5[a]	7.7 ± 0.5[b]	0.04
CL number			
Day 9	1.3 ± 0.05	1.2 ± 0.06	0.08
Day 16	1.3 ± 0.05	1.2 ± 0.06	0.09

[1] Ovulation was synchronized using Presynch + Ovsynch with or without 400 IU eCG on day 0.
[2] Cows with luteal regression after $PGF_{2\alpha}$ administration on day 0 as determined visually (ultrasonography) or by change in serum progesterone concentrations.
[3] Cows ovulating a single follicle in response to the first GnRH injection of Ovsynch.
[4] Cows ovulating more than 1 follicle in response to first GnRH injection of Ovsynch.
[5] Cows ovulating a single follicle in response to the second GnRH injection of Ovsynch.
[6] Cows ovulating more than 1 follicle in response to second GnRH injection of Ovsynch.

Table 2. Effect of equine chorionic gonadotropin (eCG) on pregnancy rates and pregnancy loss in lactating dairy cows

Item	Treatment[1] eCG	Control	P-value
Pregnancy rate per AI			
At 33 days, %	36.9	41.8	0.58
At 61 days, %	32.3	32.3	0.96
Pregnancy loss, %	4.6	9.1	0.34

[1] Ovulation was synchronized using Presynch + Ovsynch with or without 400 IU eCG on day 0.
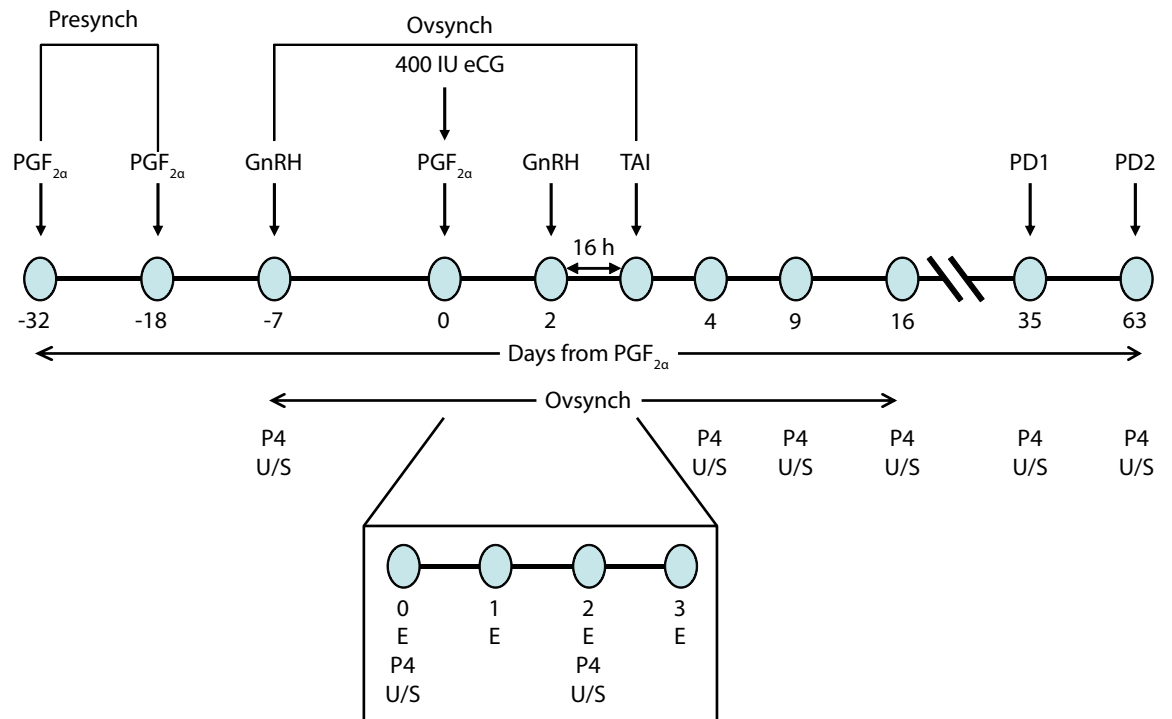
Figure 1. Experimental design.

Treatments for Presynch + Ovsynch timed artificial insemination (AI) protocol with or without administration of 400 IU equine chorionic gonadotropin (eCG) on day 0. PD = pregnancy diagnosis based on visualization of viable embryo; P4 = blood sampling and analysis of progesterone concentrations in circulating serum; E = blood sampling and analysis of estradiol concentrations in circulating serum; U/S = ultrasonography of the ovaries to assess size and changes in follicle and luteal structures.
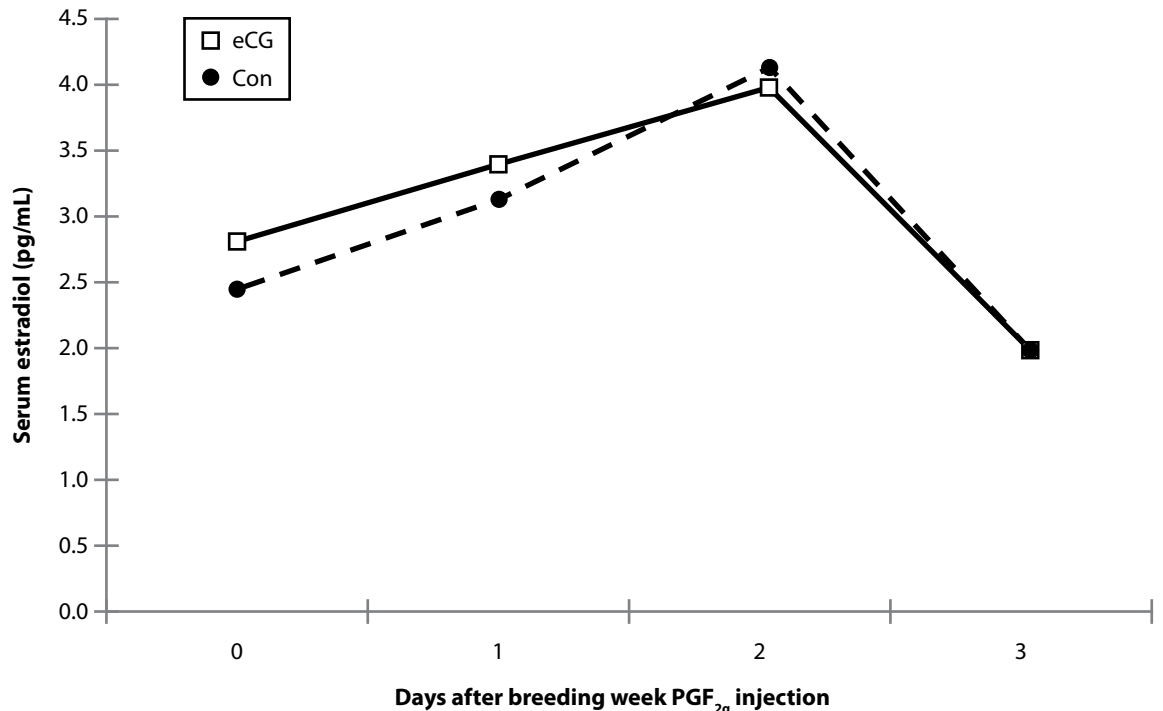

Figure 2. Pattern of serum estradiol concentrations on days 0, 1, 2, and 3 of the study.