

# Concentrations of Luteinizing Hormone and Ovulatory Responses in Dairy Cows Before Timed Artificial Insemination

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## Summary

The objective of this study was to determine the incidence of spontaneous and gonadotropin-releasing hormone (GnRH)-induced surges of luteinizing hormone (LH) and ovulatory responses in lactating dairy cows enrolled in a timed artificial insemination (TAI) protocol. Cows ( $n = 70$ ) in a single herd were assigned to one of two presynchronization protocols: Pre-10 or PG-3-G. Cows assigned to the Pre-10 treatment received 2 injections of prostaglandin  $F_{2\alpha}$  (PGF $_{2\alpha}$ ) 14 days apart (Presynch), with the second injection administered 10 days before the onset of a TAI protocol. Cows assigned to the PG-3-G treatment received an injection of prostaglandin  $F_{2\alpha}$  (PGF $_{2\alpha}$ ), then 3 days later an injection of GnRH (GnRH-1) 7 days before the onset of a TAI protocol. All cows received the first GnRH injection (GnRH-2) of the Ovsynch protocol and a PGF $_{2\alpha}$  injection 7 days later, then cows received the breeding injection of GnRH (GnRH-3) at either 56 or 72 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 LH, progesterone, and estradiol to determine why ovulatory responses to GnRH-induced LH release did not approach 100% when follicle dominance and adequate follicle size was achieved. Presynchronization administration of GnRH-1 increased the incidence of LH surges and ovulation rates in cows presynchronized with PG-3-G compared with Pre-10. Incidence of ovulation and occurrence of LH surges did not differ after GnRH-2, but more LH was released in Pre-10 than PG-3-G cows. Luteolysis, LH surge incidence, and ovulation rates were similar among the 4 treatment-time combinations after GnRH-3. Pregnancy per TAI was decreased in Pre-10 at 56 hours compared with Pre-10 at 72 hours and PG-3-G at 56 and 72 hours. We concluded that administration of GnRH 56 hours before breeding decreased pregnancy per TAI compared with administration of GnRH at 72 hours when cows were presynchronized with Pre-10. Presynchronization with PG-3-G resulted in acceptable pregnancy per TAI with GnRH administration occurring at either 56 or 72 hours before TAI. The flexibility of GnRH timing with the PG-3-G presynchronization protocol may be an advantage compared with the Pre-10 protocol for dairy cattle when programmed for a TAI at first service.

**Key words:** follicle, Ovsynch, presynchronization, timed AI

## Introduction

Timed artificial insemination (TAI) protocols provide viable alternatives to heat detection by enabling control of estrous cycles (through promoting follicular dominance, maintaining adequate follicle size, and enforcing luteolysis). The most commonly used TAI protocol in the United States dairy industry is Ovsynch.

Presynchronization before initiation of the Ovsynch protocol generally improves pregnancy per TAI (P/TAI) compared with Ovsynch alone. The standard presynchronization protocol includes 2 PGF $_{2\alpha}$  injections given 14 days apart, with the Ovsynch protocol beginning 10 to 14 days after the second PGF $_{2\alpha}$  injection. The interval between Presynch and Ovsynch has been

studied with 10 (Presynch-10), 11 (Presynch-11), 12 (Presynch-12), or 14 days (Presynch-14) between the second PGF<sub>2 $\alpha$</sub>  injection and the onset of Ovsynch. Presynch programs with intervals of 10 or 11 days have improved P/TAI compared with programs with 14 days. This increase is a consequence of an improved ovulation response to the initial gonadotropin-releasing hormone (**GnRH**) injection of Ovsynch. Other presynchronization programs involve the use of a non-breeding Ovsynch protocol (**Double-Ovsynch**), or a combination of PGF<sub>2 $\alpha$</sub>  and GnRH injections given 2 (**G-6-G**) or 3 days (**PG-3-G**) apart followed in 6 or 7 days, respectively, by enrollment in Ovsynch.

Double-Ovsynch improved P/TAI in primiparous, but not in multiparous cows compared with Presynch-12. In a recent study (Dairy Research 2012, pp. 15–21), we demonstrated that PG-3-G program increased P/TAI compared with Presynch-10 during summer and proposed that PG-3-G is a more effective presynchronization program because of its ability to induce ovulation in anovular cows and increase the number of cows with a corpus luteum and greater progesterone concentration at the onset of Ovsynch. Previous reports have stated that greater ovulatory response to the first GnRH injection of the Ovsynch protocol results in improved embryo quality and greater P/TAI. Ovulation responses to the initial GnRH injection reportedly are 67 to 79%; however, responses to the second GnRH injection are generally greater and range from 96 to 97%.

Our main research question was why ovulatory responses to both GnRH injections do not approach 100%. Administration of GnRH stimulates the release of a surge of luteinizing hormone (**LH**) and follicle-stimulating hormone (**FSH**) into circulation, which results in the ovulation of dominant follicles in 24 to 32 hours. Cows treated with GnRH during the luteal phase release less LH than cows treated during the follicular phase. Another study reported that cows with lesser progesterone concentrations had greater LH release following GnRH treatment. Although TAI programs based on the Ovsynch protocol are extensively used in the field and in research settings, relatively little is known about the characteristics of LH secretion in association with PGF<sub>2 $\alpha$</sub> -induced luteolysis and GnRH injections during these programs. The objective of this study was to determine the incidence of spontaneous and GnRH-induced surges of LH and ovulatory responses in lactating dairy cows enrolled in a TAI protocol.

## Experimental Procedures

Lactating Holstein cows ( $n = 70$ ) from the Kansas State University Dairy Teaching and Research Center were enrolled in the study. Cows were milked thrice daily and were housed in tie stalls and fed twice daily a TMR calculated to meet or exceed the nutrient requirements for a lactating Holstein cow producing 50 kg of 3.5% milk. The diet consisted of corn silage, sweet bran, cracked corn, alfalfa hay, whole cottonseed, soybean meal, vitamins, and minerals. Breeding clusters ( $n = 12$ ) were formed every 14 days as cows and heifers calved. Cows were blocked by lactation number and DIM, and blocks were assigned randomly to 2 presynchronization treatments (Figure 1). The two treatments were: (1) Pre-10 ( $n = 37$ ): two 25-mg injections of PGF<sub>2 $\alpha$</sub>  (PG-1 and PG-2) 14 days apart (Presynch); or (2) PG-3-G ( $n = 33$ ): one 25-mg injection of PG 3 days before 100  $\mu$ g GnRH (GnRH-1), with the PG injection administered at the same time as PG-2. Cows were enrolled in a TAI protocol 10 days after PG-2 (Ovsynch; injection of GnRH 7 days before [GnRH-2] and 56 or 72 hours after [GnRH-3] PG-3, with AI at 72 hours after PG-3). Inseminations occurred October 2011 through March 2012.

Body condition scores (BCS; 1 = thin and 5 = fat) were assigned on day -7 for each cow. Blood was sampled from all cows by puncture of the coccygeal vein or artery into evacuated tubes (BD Vacutainer, Franklin Lakes, NJ) or by indwelling jugular catheters. Blood was collected (Figure 1) to determine LH at: (1) GnRH-1: 0 to 80 hours after PG-2 and hourly from 72 to 78 hours (GnRH-1 at 72 hours); (2) GnRH-2: 0 to 6 hours after GnRH-2; and (3) GnRH-3: 0 to 80 hours after PG-3 and hourly from 56 to 62 or 72 to 78 hours for cows injected with GnRH at 56 or 72 hours after PG-3. Additional blood samples were collected every 12 hours after PG-3 from 0 to 72 hours 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 LH, progesterone, and estradiol by radioimmunoassay.

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 at PG-2, PG-3, GnRH-1, 2, and 3. A structural map of each ovary was drawn with the position and diameter of follicles  $\geq 5$  mm in diameter and each corpus luteum (CL), which allowed for evaluation of visual luteolysis and ovulatory response to each GnRH injection. Follicle diameter was determined by averaging the perpendicular measurements of follicular width and height using the internal calipers of the Aloka 500V. Ovulation was defined as disappearance of one or more follicles greater than  $\geq 8$  mm in diameter from an ovary, when a follicle had been recorded on the previous scan of that ovary, followed by the formation of a CL.

Pregnancy was initially diagnosed at 31 days post-TAI by ultrasonography. Pregnancy was re-confirmed at 61 days by transrectal ultrasonography. Pregnancy loss was calculated between the 2 pregnancy diagnoses.

## Results and Discussion

Cows presynchronized with PG-3-G had 3 times more induced LH surges than Pre-10 cows (Table 1) and a similar greater ovulation response to GnRH-1 than Pre-10 cows, respectively. The proportion of cows with spontaneous or no LH surge was greater in Pre-10 than PG-3-G cows, respectively (Figure 2). These results are similar to those we had previously reported, in which PG-3-G resulted in increased ovulation incidence compared with spontaneous ovulation in the Pre-10 treatment (80 vs. 53%), respectively. In addition, progesterone concentrations before the GnRH-1 injection did not differ between Pre-10 and PG-3-G cows ( $0.7 \pm 0.4$  vs.  $0.5 \pm 0.3$  ng/mL) in either the current or the previous study.

Serum LH concentrations were greater ( $P < 0.01$ ) for cows receiving Pre-10 than PG-3-G during 1 to 3 hours post-GnRH-2 (Figure 3). An induced LH surge occurred in all cows at GnRH-2 (Table 2) regardless of treatment. Concentrations of LH were greater ( $P < 0.05$ ) at 1, 2, and 3 hours after GnRH-2, but incidence of ovulation did not differ between treatments. Less LH release in the PG-3-G cows likely occurred because serum progesterone concentrations tended ( $P = 0.069$ ) to be greater in PG-3-G than Pre-10 cows before GnRH-2 injection ( $4.1 \pm 0.6$  vs.  $2.7 \pm 0.5$  ng/mL). This agrees with our previous study in which progesterone was greater in PG-3-G than Pre-10 cows, but ovulation incidences did not differ.

Serum LH concentrations were similar for cows regardless of presynchronization treatment (Figure 4) or timing of the GnRH-3 (Figure 5). More ( $P < 0.05$ ) cows had induced LH surges after GnRH-3 at 56 hours than after 72 hours (Table 3). No difference was detected in pro-

gesterone or estradiol concentrations between treatments during the sampling period for GnRH-3. At GnRH-3, ovulation was similar for Pre-10 and PG-3-G cows, regardless of time of GnRH-3 administration at 56 or 72 hours.

Pregnancy per TAI for the main effects of treatment (PG-3-G vs. Pre-10) and time of GnRH-3 (56 vs. 72 hours) did not differ, but the Pre-10, 72-hour treatment combination was less ( $P < 0.01$ ) than all other treatment combinations (Table 4).

We concluded that more PG-3-G cows had LH surges and a greater ovulation incidence at GnRH-1 compared with Pre-10. Consistent with our earlier report, cows presynchronized with the PG-3-G program had increased ovulation rates after GnRH-1 and greater ( $P = 0.069$ ) progesterone before GnRH-2 than Pre-10 cows.

**Table 1. Luteolysis, incidence of luteinizing hormone (LH) surges, and ovulation after PG-1 or PG-2 and GnRH-1**

Item	Treatment	
	Pre-10	PG-3-G
Luteolysis <sup>1</sup> ,%	56.8 (21/37)	60.6 (20/33)
LH surge <sup>2</sup> ,%	33.3 <sup>a</sup> (12/36)	100 <sup>b</sup> (33/33)
Ovulation <sup>3</sup> ,%	33.3a (12/36)	100 <sup>b</sup> (33/33)

<sup>a,b</sup> Means differ ( $P = 0.002$ ) between treatments.

<sup>1</sup>Luteolysis was determined by changes in progesterone concentration between PG-2 ( $\geq 1$  ng/mL) and 72 hours later ( $< 1$  ng/mL).

<sup>2</sup>Increase in LH concentration greater than 2 SD above baseline.

<sup>3</sup>Spontaneous ovulation or ovulation response to GnRH-1.

**Table 2. Incidence of luteinizing hormone (LH) surges and ovulation after GnRH-2**

Item	Treatment	
	Pre-10	PG-3-G
LH surge <sup>1</sup> ,%	81.1 (30/37)	90.9 (30/33)
Ovulation <sup>2</sup> ,%	62.2 (23/37)	51.5 (17/33)
Double ovulation <sup>3</sup> ,%	18.9 (7/37)	6.1 (2/33)

<sup>1</sup> Increase in LH concentration greater than 2 SD above baseline.

<sup>2</sup>Ovulation of one follicle after GnRH-2.

<sup>3</sup>Ovulation of more than one follicle after GnRH-2.

**Table 3. Luteolysis, incidence of luteinizing hormone (LH) surges, and ovulation after GnRH-3**

Item	Treatment <sup>1</sup>			
	Pre-10		PG-3-G	
	56 hours	72 hours	56 hours	72 hours
Luteolysis <sup>1</sup> , %	86.5 (32/37)		90.9 (30/33)	
LH surge <sup>2</sup> , %				
Induced	100 (19/19)	83.8 (15/18)	100 (14/14)	94.1 (16/17)
Spontaneous	0 (0/19)	16.7 (3/18)	0 (0/14)	0 (0/17)
Ovulation <sup>3</sup> , %	100 (19/19)	88.9 (16/18)	87.5 (14/16)	93.3 (14/17)
Double ovulation <sup>4</sup> , %	5.3 (1/19)	11.1 (2/18)	6.2 (1/16)	5.9 (1/17)

<sup>1</sup> Luteolysis was determined by changes in progesterone concentration between PG-2 ( $\geq 1$  ng/mL) and 72 h later ( $< 1$  ng/mL).

<sup>2</sup> Increase in LH concentration greater than 2 SD above baseline.

<sup>3</sup> Ovulation of one follicle after GnRH-2.

<sup>4</sup> Ovulation of more than one follicle after GnRH-2.

**Table 4. Pregnancy per AI after presynchronization with Pre-10 or PG-3-G and GnRH administration at 56 or 72 hours**

Item	Treatment <sup>1</sup>			
	Pre-10		PG-3-G	
	56 hours	72 hours	56 hours	72 hours
Pregnancy per AI <sup>2</sup> , %				
At 31 d	52.6 <sup>a</sup> (10/19)	22.2 <sup>b</sup> (4/18)	57.1 <sup>a</sup> (8/14)	56.3 <sup>a</sup> (9/16)
At 61 d	52.6 <sup>a</sup> (10/19)	22.2 <sup>b</sup> (4/18)	57.1 <sup>a</sup> (8/14)	53.3 <sup>a</sup> (8/15)
Pregnancy loss <sup>3</sup> , %	0 (0/19)	0 (0/18)	0 (0/14)	6.3 (1/16)

<sup>a,b</sup> Means differ ( $P < 0.002$ ) within row.

<sup>1</sup> See Figure 1.

<sup>2</sup> Determined by transrectal ultrasonography of uterine fluid and presence of viable embryo.

<sup>3</sup> Pregnancy losses were calculated between the two pregnancy diagnoses.

REPRODUCTION

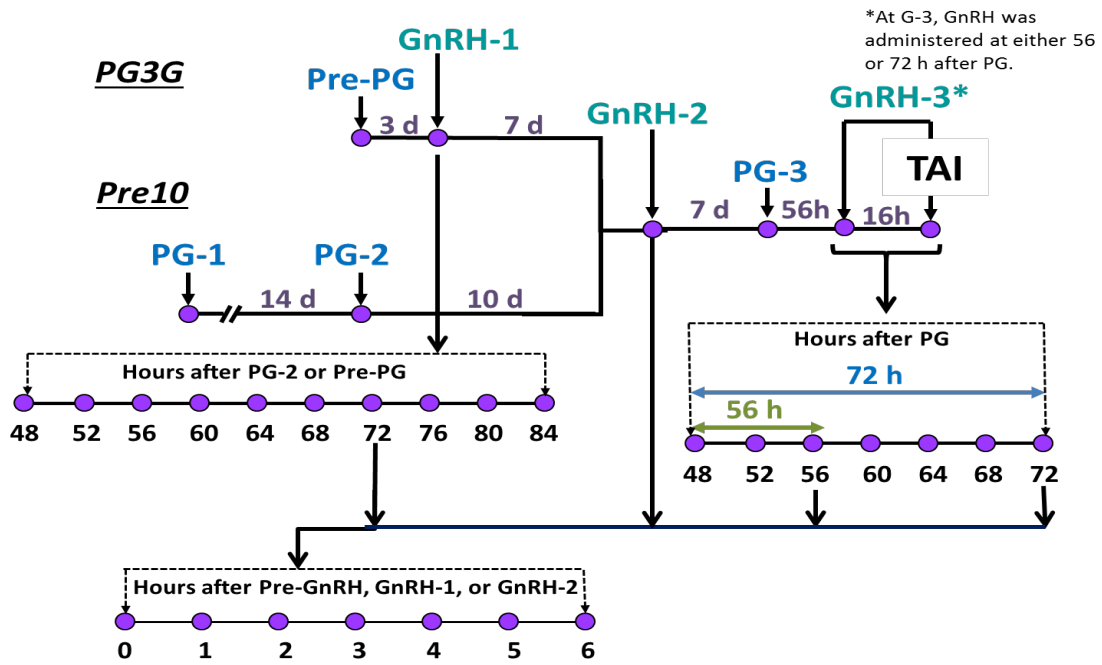


Figure 1. Experimental protocol for blood collection (PG = PGF<sub>2a</sub>, TAI = timed artificial insemination, GnRH = gonadotropin-releasing hormone).

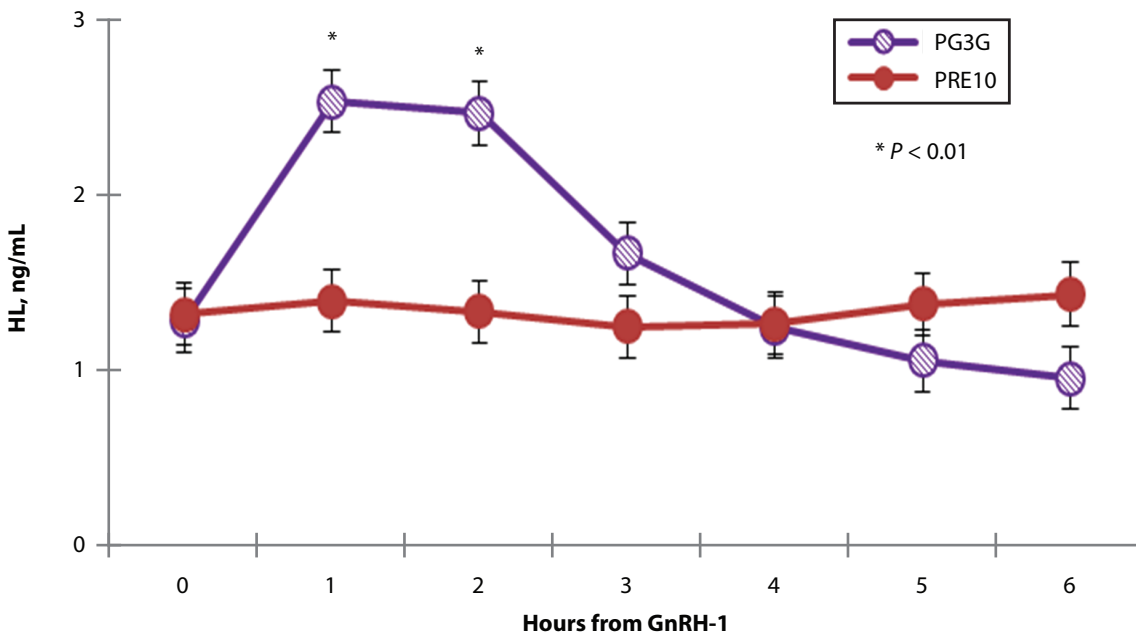


Figure 2. Pattern of serum luteinizing hormone (LH) concentrations during a period of 6 hours after GnRH-1.

### REPRODUCTION

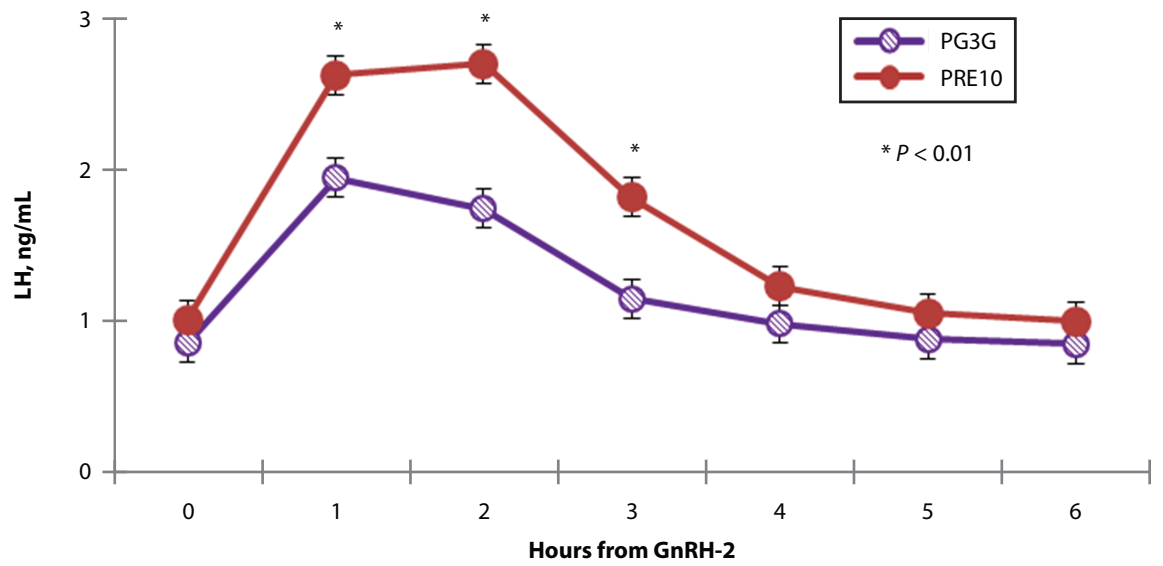


Figure 3. Pattern of serum luteinizing hormone (LH) concentrations during a period of 6 hours after GnRH-2.

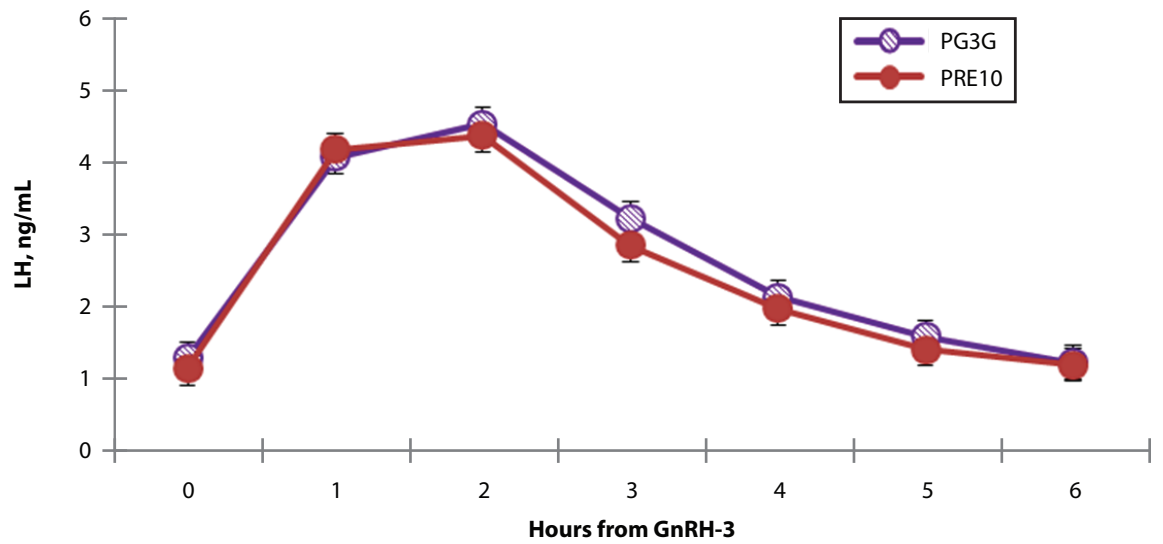


Figure 4. Pattern of serum luteinizing hormone (LH) concentrations during a period of 6 hours after GnRH-3.



# REPRODUCTION

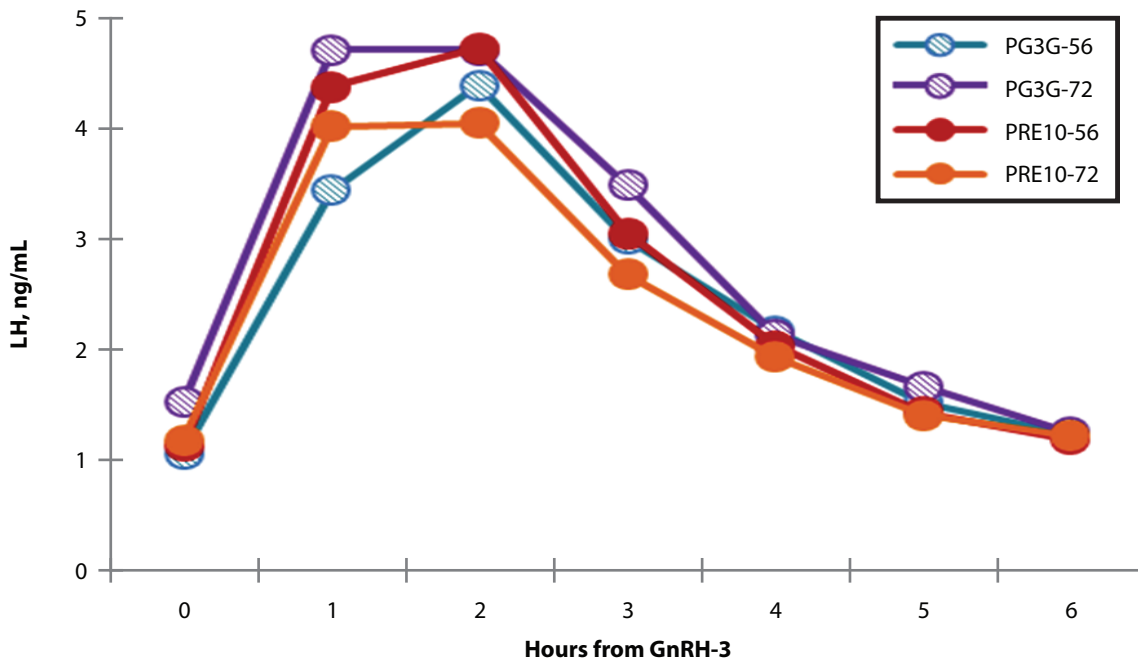


Figure 5. Pattern of serum luteinizing hormone (LH) concentrations during a period of 6 hours after GnRH-3 according to presynchronization treatment and timing of GnRH administration.