Human chorionic gonadotropin (hCG)-induced ovulation occurs later but with equal occurrence in lactating dairy cows: comparing hCG and gonadotropin-releasing hormone protocols

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Abstract. This study assessed the effects of two hormones, human chorionic gonadotropin (hCG) and gonadotropin-releasing hormone (GnRH), on ovulatory responses during different diestrous stages in lactating dairy cows. Estrous cycles of 21 cows were synchronized and were enrolled in stage 1 of the experiment. The cows were treated with a prostaglandin (PG) F₂α analog either 9 to 10 days [mid-diestrus (MD) group] or 5.5 to 6.5 days [early-diestrus (ED) group] after synchronized ovulation (day 0 = first PGF₂α administration). On day 2, the cows were administrated 250 μg GnRH or 3000 IU hCG. Ovulation was determined every 2 h from 24 to 36 h after GnRH or hCG administration, and then every 4 h up to 72 h until ovulation. Cows in stage 2 were administered these treatments in the reverse order. The results indicated that average ovulation times in cows treated with GnRH in the MD group (GnRH-MD group) and cows treated with GnRH in the ED group (GnRH-ED group) were 30.0 ± 1.0 h and 28.8 ± 0.4 h, respectively. However, ovulation times for cows treated with hCG in the MD group (hCG-MD group) and cows treated with hCG in the ED group (hCG-ED group) were 35.8 ± 4.6 h and 32.8 ± 2.2 h, respectively, and ovulation occurred significantly later in the hCG-treated groups than in the GnRH-treated groups. In summary, we found that hCG-induced ovulation occurred later than GnRH-induced ovulation regardless of different diestrous periods; however, the two treatments did not differ in terms of percentage of ovulation.

Key words: Gonadotropin-releasing hormone (GnRH), Human chorionic gonadotropin (hCG), Ovulation time

This study aimed to investigate the effects of hCG on ovulatory...
responses during mid- and early-diestrus. We hypothesized that hCG-induced ovulation would occur earlier than GnRH-induced ovulation, but the percentage of ovulation would be similar regardless of the different diestrous periods.

Materials and Methods

Animals and management

This study, comprising about 60 Holstein cattle, was conducted on a dairy farm at the National Chung Hsing University (subtropical Taiwan) between August 2017 and April 2018. All the cows were housed in groups in free-stall barns with a slatted floor and equipped with free-stall bed mats, overhead fans, and a sprinkler system. Access to an outdoor shaded exercise yard was also available. Lactating dairy cows were fed with a total mixed ration (TMR) twice daily ad libitum. The diet (TMR) consisted of approximately 58% hay (Bermuda and alfalfa) and 42% concentrate (commercial milking cow concentrate, steam-flaked corn, and wheat bran), which was mixed with minerals (dicalcium phosphate and magnesium oxide) and vitamin E. Fresh water was provided ad libitum. All lactating dairy cows were milked twice daily, and the average daily milk yield was 25 kg per cow. During the experimental period, temperature and humidity were recorded at 0600 h, 1200 h, 1800 h, and 2400 h, and the thermal-humidity index (THI) was calculated. All the procedures were approved by the Animal Care and Use Committee for Biotechnology of the National Chung Hsing University (IACUC No. 106-058). The lactating dairy cows, between 50 and 70 days in milk (DIM) and with normal uterus involution and ovarian function was enrolled for this experiment. The parity of the cows ranged from 1 to 4, except for two cows that gave birth six and seven times, respectively [average parity: 2.9 ± 1.6 (mean ± SD)]. The body condition score (BCS) of the cows ranged from 2.5 to 3.25 based on a 5-point scale [21], and the average BCS was 3.03 ± 0.26.

Experimental design

A total of 21 lactating dairy cows were subjected to synchronization of the estrous cycle employing the modified Ovsynch-48 protocol, and the following experimental protocol was conducted on the animals after successful synchronization (Fig. 1). The modified Ovsynch–48 protocol involved administration of 250 μg GnRH (100 μg/ml Fertagyl/gonadorelin; Intervet Deutschland, Unterschleiβheim, Germany) intramuscularly (im) to cows having random estrous cycle; 7 days later, the cows received 375 μg of PGF₂α (250 μg/ml Estrumate/cloprostenol sodium; Intervet Deutschland), im (the time of first prostaglandin (PG) F₂α injection during synchronization; SynPG). A second dose of PGF₂α (250 μg) was administered after 24 h, and another dose of GnRH (250 μg) was administrated 48 h after the first dose of PGF₂α (SynG2). If the cows were not synchronized, by presenting no signs of complete luteolysis/ovulation but were still between 50 and 70 DIM, the process of synchronization was repeated using a modified Ovsynch-48 protocol.

After successful synchronization, we first subdivided the cows in two groups: the mid-diestrus group (MD group; 9 to 10 days after ovulation) and the early-diestrus group (ED group; 5.5 to 6.5 days after ovulation). In the MD group, cows were enrolled in stage 1 (DIM: 76.6 ± 9.0) of the experimental protocol 9 to 10 days after ovulation of synchronization. The time of the first PGF₂α administration was defined as day 0 of stage 1. On days 0 and 1, cows were treated with 375 and 250 μg PGF₂α, respectively. On day 2, the cows were administered im with 250 μg GnRH (GnRH-MD group) or 3000
IU hCG (1,500 IU/ampule Pregnyl/chorionic gonadotrophin; N.V. Organon, Netherlands) (hCG-MD group). If both ovulation within 72 h after GnRH or hCG administration as well as complete luteolysis occurred, the cows were further enrolled in stage 2 of the experimental protocol. Cows with partial luteolysis or absence of ovulation were not assigned to the next stage. During 9 to 10 days after ovulation of stage 1, the treatment protocol in stage 1 was reversed for cows in stage 2 (DIM: 91.0 ± 9.6) — i.e., cows treated with GnRH in stage 1 were administered hCG in stage 2 and vice versa.

In the ED group, all procedures were essentially the same as those used in the MD group except that the cows were treated with the first dose of PGF$_{2a}$ between 5.5 and 6.5 days after ovulation of synchronization and stage 1. Cows treated with GnRH were allocated to the GnRH-ED group and the others to hCG-ED group. In stages 1 and 2, the average DIM in the ED group was 69.7 ± 4.2 and 80.1 ± 2.5, respectively.

**Ultrasound examination for CL area, follicle diameter, and ovulation**

Transrectal B-mode ultrasonography of the ovaries was performed using a portable scanner equipped with a 7.5-MHz linear-array transducer (SonoSite Ultrasound System; SonoSite, Bothell, WA, USA) at SynPG, 24 h after the second GnRH injection of the synchronization stage, and once a day from days 0 to 3 of stages 1 and 2. The method used to measure the longitudinal and transverse axes of the CL and to calculate the remaining CL area has been described previously [4].

The follicle diameter was determined by calculating the average of its longitudinal and transverse axes. The maximum follicle diameters at 24 h after the second GnRH dose of the synchronization stage and on day 3 of stages 1 and 2 were used to quantify the preovulatory follicle diameters. Ovulation was identified by the disappearance of preovulatory follicles followed by the appearance of a new CL at the same site in the ovary. During synchronization, ovulation was determined at 24 h and 32 h after the administration of second GnRH dose. During stages 1 and 2, ovulation was verified every 2 h from 24 to 36 h after GnRH or hCG injections, and then every 4 h up to 72 h until ovulation occurred. The time of disappearance of the preovulatory follicles was defined as the ovulation time.

**Blood sampling and hormone analysis**

Blood samples were collected from the coccygeal vessels and immediately refrigerated. The samples were then centrifuged (1,300 × g, 10 min), and the serum was harvested and stored at −20°C until the assay. Blood sampling for analysis of P$_4$ concentrations was performed for all the cows at SynPG, at 24 h after the second dose of GnRH of the synchronization stage and on days 0 and 3 of stages 1 and 2. Serum P$_4$ concentrations were measured using an enzyme immunoassay kit (Progesterone ELISA, Demeditec Diagnostics GmbH, Kiel, Germany), with a sensitivity of 0.045 ng/ml. The average intra- and inter-assay coefficients of variation were 6.42% and 6.63%, respectively.

During stages 1 and 2, blood samples for analysis of LH and hCG concentrations were taken before and at every 1 h from 1 to 6 h after GnRH or hCG injections (Fig. 1). Serum LH concentrations were determined using an ELISA sandwich assay kit (LH DETECT for bovines with tetramethylbenzidine substrate; ReproPharm Vet, Nouzilly, France) in all the cows, with a sensitivity of 0.1 ng/ml. The intras- and inter-assay coefficients of variation for this assay were 2.5% and 6.0%, respectively. Serum hCG concentrations in 20 cows (GnRH-MD group = 4, hCG-MD group = 5, GnRH-ED group = 5, and hCG-ED group = 6) were estimated using an enzyme-linked fluorescent assay kit (VIDAS HCG, BioMérieux, Marcy-l’Étoile, France) on a MiniVidas automated analyzer (BioMérieux). The measurement range of this kit was 2 to 1500 mIU/ml, and the detection limit was 2 mIU/ml. The average intra- and inter-assay coefficients of variations were 5.2% and 5.6%, respectively.

**Markers of complete luteolysis and successful synchronization**

Complete luteolysis was identified by either a P$_4$ concentration < 1 ng/ml or a remaining CL area < 50% at 24 h after the second dose of GnRH of the synchronization stage and on day 3 of the stages 1 and 2 [4]. Successful synchronization was defined using the following three criteria: 1) P$_4$ concentration > 1 ng/ml with accompanying CL formation and at least one follicle with a diameter ≥ 8 mm at SynPG, 2) complete luteolysis at 24 h after the second dose of GnRH, and 3) ovulation of one or more follicles at 32 h after the second GnRH administration.

**Milk production**

Milk yield in each cow was recorded from days 0 to 3 of stages 1 and 2. Average milk production was calculated for each animal in stages 1 (29.3 ± 5.6 l/day) and 2 (28.4 ± 5.8 l/day).

**Statistical analysis**

Statistical analysis of the data was performed using the SAS software - version 9.4 (SAS Institute, Raleigh, NC, USA). Statistical significance was set at P < 0.05. We considered 0.05 ≤ P < 0.10 as trends. Given the limited sample size and the non-normality of the distribution of the data, we performed group comparisons to test for differences in P$_4$ concentration, remaining CL area, follicular diameter on day 0, preovulatory follicle diameter, ovulation time, DIM, BCS, parity, average THI, and average milk production using the Kruskal-Wallis test (in GnRH-MD, hCG-MD, GnRH-ED, and hCG-ED groups) or the Wilcoxon rank-sum test (GnRH vs. hCG and MD vs. ED). In addition, a post-hoc test for the Kruskal-Wallis analysis was conducted as described in a previous study [22]. Differences in the percentages of complete luteolysis, overall percentages of ovulation, percentages of ovulation within 36 h of GnRH or hCG injection, and percentages of multiple ovulation (number of cows having more than one ovulated follicle/number of cows ovulating) were analyzed using the chi-square test and the Fisher’s exact test for comparison among four groups and between two groups, respectively. Differences in LH and hCG concentrations measured at various time points were analyzed using repeated measures ANOVA, and the Bonferroni’s method was applied to perform multiple comparison.
Results

Synchronization

Synchronization was achieved for 24 estrous cycles from 21 cows. There were three cows that underwent the modified Ovsynch-48 protocol twice. The results of synchronization are shown in Table 1. Complete luteolysis could be identified in all the cows, and ovulation occurred in 20 synchronized cycles. The overall percentage of the synchronized cycles was 83.3% (20/24).

Stages 1 and 2

Before initiating the stage 1 experiment, one cow was excluded because of acute mastitis. In this study, because the sample size in the stages 1 or 2 of each treatment group was too small (n = 3 to 5), a sequence effect (i.e., at stages 1 and 2) on the percentages of complete luteolysis and ovulation was tested. Statistical analysis showed no sequence effect on these parameters (data not shown).

Except for five cows detected with more than one CL, all the remaining animals had only one CL. On day 0, the P₄ concentrations were higher in GnRH-MD than in the GnRH-ED and hCG-ED groups; however, no significant differences could be detected for P₄ concentration on day 3 (Table 2). On average, the values of the remaining CL area in the GnRH-MD group from day 1 to 3 were lower than those in the GnRH-ED group. Although there was each one cow with partial luteolysis in the GnRH-ED and hCG-ED groups, the percentage of complete luteolysis was not statistically different among the four groups.

Mean LH profiles throughout the sampling period were affected by different groups, time periods, and a group by time interaction (all P < 0.05). During hours 1 to 3, higher LH concentrations were found in the GnRH-MD and GnRH-ED groups than those in the other two groups. Peak LH concentrations occurred at 2 h after GnRH administration in the GnRH-MD and GnRH-ED groups; the concentrations then decreased until they returned the baseline at 5 h after treatment. With the exception of one cow in the hCG-ED group that showed a spontaneous LH surge and had a similar profile to that observed for the GnRH-MD and GnRH-ED groups, the LH concentration of the cows in the hCG-ED groups remained at baseline levels (Fig. 2).

Mean hCG profiles throughout the sampling period were affected by different groups, time periods, and a group by time interaction.
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![Graph](image1.png)

![Graph](image2.png)

**Fig. 2.** Luteinizing hormone (LH) concentrations in lactating dairy cows receiving treatment in the GnRH-MD, hCG-MD, GnRH-ED, and hCG-ED groups. GnRH, gonadotropin-releasing hormone; GnRH-MD, treated with GnRH in the mid-diestrus group; GnRH-ED, treated with GnRH in the early-diestrus group; hCG-MD, treated with human chorionic gonadotropin (hCG) in the mid-diestrus group; hCG-ED, treated with hCG in the early-diestrus group. Data are shown as mean ± SEM. The P values of different groups, time periods, and a group by time interaction were < 0.05.

**Fig. 3.** Human chorionic gonadotropin (hCG) concentrations in lactating dairy cows receiving treatment in the GnRH-MD, hCG-MD, GnRH-ED, and hCG-ED groups. GnRH-MD, treated with gonadotropin-releasing hormone (GnRH) in the mid-diestrus group; GnRH-ED, treated with GnRH in the early-diestrus group; hCG-MD, treated with hCG in the mid-diestrus group; hCG-ED, treated with hCG in the early-diestrus group; Data are shown as mean ± SEM. The P values of different groups, time periods, and a group by time interaction were < 0.05.

**Table 3.** Time of ovulation after data from stages 1 and 2 were pooled together

| Group             | Ovulation time (h) after GnRH or hCG injection |
|-------------------|-----------------------------------------------|
|                   | ≤ 28  ≤ 30  ≤ 32  ≤ 34  ≤ 36  > 36  no ovulation |
| GnRH-MD (n = 9)   | 4      0      2      1      0      0      2 |
| hCG-MD (n = 9)    | 0      4      2      1      0      1      1 |
| GnRH-ED (n = 8)   | 5      3      0      0      0      0      0 |
| hCG-ED (n = 8)    | 1      3      3      0      0      1      0 |

hCG, human chorionic gonadotropin; GnRH, gonadotropin-releasing hormone; GnRH-MD, treated with GnRH in the mid-diestrus group; GnRH-ED, treated with GnRH in the early-diestrus group; hCG-MD, treated with hCG in the mid-diestrus group; hCG-ED, treated with hCG in the early-diestrus group; > 36: ovulation occurred between 40 h and 72 h.

(all P < 0.05). Except for 0 and 1 h, the hCG concentrations were higher in the hCG-MD and hCG-ED groups than in the other two groups. The hCG concentrations between 0 and 6 h in the GnRH-MD and GnRH-ED groups were 2 mIU/ml below the detection limit. We observed an increase of the hCG concentration during the first three hours in the hCG-MD and hCG-ED groups, followed by a slight deceleration in the rate of increase (Fig. 3).

As shown in Table 3, all the cows ovulated between 28 and 32 h after GnRH administration (with the exception of one cow that ovulated at 34 h and two cows that did not ovulate). One cow, which was initially identified with partial luteolysis, ovulated at 28 h. However, ovulation occurred between 30 and 32 h in the majority of cows treated with hCG. Two cows were observed to ovulate at 34 h and 68 h, and one cow that did not ovulate in the hCG-MD group. In the hCG-ED group, one cow with a spontaneous LH surge ovulated at 28 h, and one cow ovulated at 48 h. In the cow showing partial luteolysis, ovulation occurred at 32 h. Overall, the occurrence of ovulation in the hCG-MD group was later than that in the GnRH-ED group (Table 2). Although we excluded data from the cows ovulating later than 36 h after GnRH or hCG administration, the ovulation time still tended to be different between these two groups (P = 0.06). The average follicle diameter on day 0 was smaller in the GnRH-ED group than in the GnRH-MD group; however, the mean values for preovulatory follicle diameters varied between 15 and 17 mm and were not different among the four groups. Furthermore, the overall percentage of ovulation and the percentage of ovulation within 36 h of treatment were not different among the groups. There was one cow in the hCG-MD group, two in GnRH-ED group, and one in hCG-ED group that showed multiple ovulation; however, the percentage of multiple ovulation was not different among groups.
Comparisons between the GnRH and hCG groups

With respect to luteolysis, there was no difference in P₄ concentration or in the remaining CL area between the GnRH and hCG groups (Table 4). The percentage of complete luteolysis was 94.1% for both the groups. Regarding ovulatory responses, excluding ovulation time, there were no significant differences between the GnRH and hCG groups in terms of follicle diameter on day 0, preovulatory follicle diameter, the percentage of overall ovulation, the percentage of ovulation within 36 h, and the percentage of multiple ovulation. Collectively, ovulation in the GnRH group occurred earlier than in the hCG group. After excluding two cows that ovulated later than 36 h post-treatment, we observed that the period between GnRH injection and ovulation in the GnRH group was shorter than that in the hCG group (29.3 ± 0.5 h vs. 30.9 ± 0.4 h, P = 0.0240; mean ± SEM).

Comparisons between the MD and ED groups

We did not observe any significant differences in ovulatory responses between the MD and ED groups, except for differences in follicle diameters on day 0, which were significantly smaller in the ED group than in the MD group (Table 5). With respect to the CL, the P₄ concentration in the MD group was higher on day 0 than in the ED group. Although there were no significant differences between the two groups in terms of P₄ concentrations on day 3, the remaining CL area between days 1 and 3 were lower in the MD group than in the ED group. The percentages of complete luteolysis were 100% and 87.5% in the MD and ED groups, respectively; however, this difference was not statistically significant.

| Table 4. Luteolytic and ovulatory parameters in the GnRH and hCG groups |
| Parameters | GnRH (n = 17) | hCG (n = 17) | P |
|------------|--------------|--------------|---|
| P₄ on day 0 (ng/ml) | 6.52 ± 0.67 | 6.39 ± 0.74 | 0.7977 |
| P₄ on day 3 (ng/ml) | 0.63 ± 0.21 | 0.61 ± 0.13 | 0.2431 |
| Remaining CL area on day 1 (%) | 70.8 ± 4.1 | 71.5 ± 3.5 | 0.6088 |
| Remaining CL area on day 2 (%) | 52.4 ± 3.3 | 51.9 ± 2.1 | 0.7846 |
| Remaining CL area on day 3 (%) | 35.6 ± 2.6 | 38.1 ± 2.9 | 0.4540 |
| Complete luteolysis (%) | 94.1 (16/17) | 94.1 (16/17) | 1.0000 |
| Follicle diameter on day 0 (mm) | 13.8 ± 0.9 | 14.7 ± 0.7 | 0.6729 |
| Preovulatory follicle diameter (mm) | 16.0 ± 0.8 | 16.5 ± 0.7 | 0.6296 |
| Ovulation time (h) | 29.3 ± 0.5 b | 34.3 ± 2.5 a | 0.0098 |
| Ovulation (%) | 88.2 (15/17) | 94.1 (16/17) | 1.0000 |
| Ovulation within 36 h (%) | 88.2 (15/17) | 82.4 (14/17) | 1.0000 |
| Multiple ovulation (%) | 13.3 (2/15) | 12.5 (2/16) | 1.0000 |

CL, corpus luteum; P₄, progesterone; GnRH, cows treated with gonadotropin-releasing hormone; hCG, cows treated with human choriionic gonadotropin. Data are shown as mean ± SEM. Different superscript letters indicate statistically significant difference between groups in the same row; Categorical and continuous data were tested by the Fisher’s exact test and the Wilcoxon rank-sum test, respectively.

| Table 5. Luteolytic and ovulatory parameters in the MD and ED groups |
| Parameters | MD (n = 18) | ED (n = 16) | P |
|------------|------------|------------|---|
| P₄ on day 0 (ng/ml) | 8.05 ± 0.62 a | 4.66 ± 0.48 b | 0.0009 |
| P₄ on day 3 (ng/ml) | 0.46 ± 0.03 | 0.81 ± 0.26 | 0.5500 |
| Remaining CL area on day 1 (%) | 65.2 ± 2.9 b | 77.9 ± 4.1 a | 0.0126 |
| Remaining CL area on day 2 (%) | 47.0 ± 1.8 b | 57.9 ± 3.1 a | 0.0126 |
| Remaining CL area on day 3 (%) | 31.8 ± 1.9 b | 42.5 ± 3.0 a | 0.0082 |
| Complete luteolysis (%) | 100 (18/18) | 87.5 (14/16) | 0.2139 |
| Follicle diameter on day 0 (mm) | 15.8 ± 0.7 a | 12.8 ± 0.8 b | 0.0208 |
| Preovulatory follicle diameter (mm) | 16.5 ± 0.6 | 16.0 ± 0.9 | 0.8927 |
| Ovulation (%) | 83.3 (15/18) | 100.0 (16/16) | 0.2299 |
| Ovulation within 36 h (%) | 77.8 (14/18) | 93.8 (15/16) | 0.3402 |
| Multiple ovulation (%) | 6.7 (1/15) | 18.8 (3/16) | 0.5996 |

CL, corpus luteum; P₄, progesterone; MD, all cows treated with gonadotropin-releasing hormone (GnRH) or human choriionic gonadotropin (hCG) in the mid-diestrus; ED, all cows treated with GnRH or hCG in the early-diestrus. Data are shown as mean ± SEM. Different superscript letters indicate statistically significant difference between groups in the same row; Categorical and continuous data were tested by the Fisher’s exact test and the Wilcoxon rank-sum test, respectively.
Discussion

In this study, we aimed to investigate the effects of hCG induction during mid- and early-diestrus on ovulatory responses in lactating dairy cows. Human chorionic gonadotropin acts directly on LH receptors and thus induces ovulation independent of the pituitary [6]; therefore, we hypothesized that ovulation after hCG administration may occur earlier than ovulation after GnRH administration. Contrary to our initial predictions, our results suggest that the hCG-induced ovulation time was 2 to 4 h later than GnRH-induced ovulation. Previous studies have reported an LH surge occurs 1 or 2 h after a GnRH pulse [23–25], and our findings were in line with these studies. Additionally, the ovulation time determined in our study had a number of similarities with that reported by Giordano et al. (2012) [3] and replicated our earlier data [4]. Other researchers have demonstrated that the serum concentration of hCG was above the baseline even at 66 h in cows treated with 3000 IU hCG (1000 IU iv and 2000 IU im) [26]. Another study reported that hCG concentration reaches a maximum at 4 h after intramuscular hCG administration (3300 IU), remains at plateau between 4 and 12 h, and decreases to baseline maximum at 4 h after intramuscular hCG administration (3300 IU), im) [26]. Another study reported that hCG concentration reaches a maximum at 66 h in cows treated with 3000 IU hCG (1000 IU iv and 2000 IU im) [26]. Another study reported that hCG concentration reaches a maximum at 66 h in cows treated with 3000 IU hCG (1000 IU iv and 2000 IU im) [26].

Interestingly, we observed that one follicle in the GnRH-MD group with 17.6 × 14.0 mm diameter on day 3 was detected at 28 h after GnRH injection and subsequently disappeared at 30 h. However, since development of a CL was not detected 5 days later, it was identified as a case of anovulation. In addition to endothelial cells, fibroblasts, and immune cells, the CL is composed of large and small luteal cells [34], which are transformed from granulosa and theca cells, respectively [35]. Apoptosis of granulosa cells has been demonstrated when the follicles undergo atresia [36, 37]. From our observations, we can speculate that the follicle described above underwent a process of gradual atresia with apoptosis of the granulosa cells instead of being transformed into large luteal cells.

The percentages of complete luteolysis were 100% in both the GnRH-MD and hCG-MD groups, which is consistent with our previous findings [4, 20] and provides evidence once again towards the advantage of using two low doses of PGF2α for the complete regression of mature CL. In this study, we found two cows with partial luteolysis as indicated by parameters, such as P4 concentration above 1 ng/ml and remaining CL area more than 50% in the ED group during stage 1. Valdecarbes-Torres and co-workers (2012) showed that a single standard dose or double dose of PGF2α for CL aged 5.5 days resulted in 80% and 100% of complete luteolysis, respectively [38]. Considering a period of seven days between the first GnRH and PGF2α in the Ovsynch protocol [1] and 28 to 30 h ovulation time after GnRH administration [3], we expected to achieve a similar effect using two low doses of PGF2α. Although we achieved a high proportion (87.5%) of complete luteolysis in the ED group, partial luteolysis occurred in some animals, which were consequently excluded from the next stage of the experiment. To prevent partial luteolysis and non-ovulation, we suggest that the optimal interval from ovulation to the first PGF2α treatment should be designed to fall on any day between 6.5 and 9 days in further experiments having a similar design.

In summary, we provide evidence that hCG-induced ovulation occurred later than GnRH-induced ovulation, but the percentages of
ovulation did not differ between hCG and GnRH treatment during mid- and early-diestrus. Even with high percentages of ovulation and complete luteolysis, risk of non-ovulation and partial luteolysis may exist when an ovulation protocol is started in the early and middle phases of diestrus, respectively.

Acknowledgments

The authors thank the farm employees for technical assistance.

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