

# JOURNAL OF ANIMAL SCIENCE

*The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science*

## **Factors affecting preovulatory follicle diameter and ovulation rate after gonadotropin-releasing hormone in postpartum beef cows. Part I: Cycling cows**

J. A. Atkins, M. F. Smith, K. J. Wells and T. W. Geary

*J Anim Sci* 2010.88:2300-2310.

doi: 10.2527/jas.2009-2531 originally published online Mar 12, 2010;

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://jas.fass.org/cgi/content/full/88/7/2300>



**American Society of Animal Science**

[www.asas.org](http://www.asas.org)

# Factors affecting preovulatory follicle diameter and ovulation rate after gonadotropin-releasing hormone in postpartum beef cows.

## Part I: Cycling cows<sup>1</sup>

J. A. Atkins,\* M. F. Smith,\* K. J. Wells,\*<sup>2</sup> and T. W. Geary†<sup>3</sup>

\*Division of Animal Sciences, University of Missouri, Columbia 65211;  
and †USDA-ARS, Fort Keogh Livestock and Range Research Laboratory, Miles City, MT 59301

**ABSTRACT:** Cows induced to ovulate small dominant follicles were reported to have reduced pregnancy rates compared with cows that ovulated large follicles. The reason for the presence of small dominant follicles at the time of GnRH-induced ovulation in timed AI protocols is unknown. The objectives of this experiment were to examine the role of day of the estrous cycle at initiation of treatment on ovulation after the first GnRH injection (GnRH1) and associated effects on growth rate and final size of the ovulatory follicle at the second GnRH injection (GnRH2), serum concentrations of estradiol at GnRH2, and subsequent luteal concentrations of progesterone in suckled beef cows. Estrous cycles of cows were manipulated to be at 1 of 5 specific days of the cycle (d 2, 5, 9, 13, and 18, d 0 = estrus; n = 12 per treatment group) at the beginning of the CO-Synch protocol (GnRH1 on d -9, PGF<sub>2α</sub> on d -2, and GnRH2 on d 0). Day of the estrous cycle at GnRH1 did not affect the size of the preovulatory follicle or the proportion of cows ovulating at GnRH2 ( $P = 0.65$  and  $0.21$ , respectively). When all cows were included in the analysis, cows that ovulated after GnRH1 had similar follicle size at GnRH2 compared with cows

that did not ovulate after GnRH1 (11.4 and 10.4 mm, respectively;  $P = 0.23$ ). When only cows that could ovulate after GnRH1 (excluding cows treated on d 2) were included in the analysis, cows that ovulated to GnRH1 had a larger follicle at GnRH2 than cows that did not ovulate after GnRH1 (11.4 and 9.5 mm, respectively;  $P = 0.04$ ). Follicle growth from d -5 to 0 was similar between cows that ovulated after GnRH1 and cows that did not (1.01 vs. 0.89 mm/d, respectively;  $P = 0.75$ ). There was a tendency for faster follicle growth rate in cows that ovulated a large follicle (>11 mm) compared with cows that ovulated a small follicle ( $\leq 11$  mm; 1.01 vs. 0.86 mm/d, respectively;  $P = 0.07$ ). Serum concentrations of estradiol at GnRH2 and progesterone after ovulation were reduced in cows that ovulated small follicles compared with cows that ovulated large follicles ( $P = 0.006$  and  $0.005$ , respectively). In summary, day of the estrous cycle at initiation of synchronization did not affect ovulatory follicle size, but follicle growth rates affected the size of the follicle at GnRH2. Cows that ovulated a small follicle had reduced serum concentrations of estradiol at GnRH2 and progesterone after ovulation.

**Key words:** beef cattle, estradiol, follicle diameter, follicle growth, ovulation rate, progesterone

©2010 American Society of Animal Science. All rights reserved.

J. Anim. Sci. 2010. 88:2300–2310  
doi:10.2527/jas.2009-2531

## INTRODUCTION

Ovulation of a small, and presumably physiologically immature, dominant follicle reduced pregnancy rates

(Lamb et al., 2001; Vasconcelos et al., 2001; Perry et al., 2005) and increased late embryonic or fetal loss (Perry et al., 2005) in beef and dairy cattle. Factors affecting ovulatory follicle size, or the mechanisms by

<sup>1</sup>This project was supported by National Research Initiative Competitive Grant No. 2006-35203-17284 from the USDA Cooperative State Research, Education, and Extension Service (Washington, DC). Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA, Montana Agriculture Experiment Station (Bozeman), or the authors and does not imply its approval to the exclusion of other products that may also be suitable. The USDA, ARS, Northern Plains Area, is an equal opportunity/affirmative action employer. All agency services are avail-

able without discrimination. The authors acknowledge Teva Animal Health (St. Joseph, MO) and Pfizer Animal Health (New York, NY) for their support of this research.

<sup>2</sup>Current address: Ohio Agricultural Research and Development Center (OARDC), Jackson Agricultural Research Station, Jackson, OH 45640.

<sup>3</sup>Corresponding author: Tom.Geary@ars.usda.gov

Received September 25, 2009.

Accepted March 5, 2010.

which ovulatory follicle size affect fertility have not been determined. Administration of a first GnRH injection (**GnRH1**) 7 d before PGF<sub>2α</sub> and a second GnRH injection (**GnRH2**) has been used to breed beef cattle by appointment (CO-Synch; Geary and Whittier, 1998). The GnRH1 injection is expected to ovulate a dominant follicle and initiate a new follicular wave so that a viable preovulatory follicle is present at timed AI. It is logical that small dominant follicles present at the time of GnRH2 could result from failure to ovulate a dominant follicle and initiate a new follicular wave with GnRH1. Failure to initiate a new follicular wave with GnRH1 did not affect follicle diameter at GnRH2 or fertility in beef heifers (Atkins et al., 2008). Alternatively, slower growth rate of the follicle could result in a small dominant follicle at GnRH2. In the present study, estrous cycles of beef cows were manipulated so that cows were on specific days of the estrous cycle at the beginning of the study. These days were selected based on prediction of the presence of a dominant follicle that either would or would not respond to GnRH1 and ovulate a first-, second-, or third-wave dominant follicle in response to GnRH2.

Our hypothesis was that day of the estrous cycle at GnRH1, and thus ovulatory response to GnRH1, would affect growth rate, health, and diameter of the ovulatory follicle at the GnRH-induced ovulation for timed AI. Our objective was to determine how day of the estrous cycle at GnRH1 would affect ovulation after GnRH1 and the associated effects on size and physiological maturity of the dominant follicle at GnRH2 in cycling beef cows.

## MATERIALS AND METHODS

All protocols and procedures were approved by the Fort Keogh Livestock and Range Research Laboratory Animal Care and Use Committee.

### *Animal Handling*

Postpartum suckled beef cows ( $n = 60$ ) that had resumed cyclicity [based on electronic observation of estrus (described below) and formation of a corpus luteum via ultrasound] were assigned to 1 of 5 treatment groups based on age and days postpartum. The treatment groups ( $n = 12$  per group) were defined as the day of the estrous cycle at the beginning of the CO-Synch protocol [d 2, 5, 9, 13, and 18 treatment groups (d 0 = estrus), referred to as **D2**, **D5**, **D9**, **D13**, and **D18**, respectively]. These days were selected based on prediction of the presence of a dominant follicle that either would or would not respond to GnRH1 and ovulate a first-, second-, or third-wave dominant follicle in response to GnRH2 (Ginther et al., 2001). All cows were treated with the CO-Synch protocol (GnRH1 on d -9, followed by PGF<sub>2α</sub> on d -2 and GnRH2 on d 0), except that cows were not bred.

### *Presynchronization*

Cows were synchronized to be on their assigned day of the cycle using a controlled internal drug-releasing device (**CIDR**; EAZI-Breed CIDR containing 1.38 g of progesterone, Pfizer Animal Health, New York, NY) for 7 d with an injection of GnRH at insertion and PGF<sub>2α</sub> at CIDR removal. Cows that displayed estrus ( $\pm 12$  h) on the same day were included in the treatment groups.

### *Estrous Detection*

The HeatWatch Estrous Detection System (Cow-Chips LLC, Manalapan, NJ) was used to monitor estrus during the presynchronization period and throughout the experiment. Estrus was defined as 3 mounts lasting longer than 2 s per mount within a 4-h period.

### *Transrectal Ultrasonography*

Ovarian structures were monitored using an Aloka 500V ultrasound instrument with a 7.5-MHz transducer (Aloka, Wallingford, CT). Follicles  $\geq 5$  mm in diameter and the presence of a corpus luteum were recorded. Follicle diameter was measured at the widest point and at a right angle to the first measurement. The follicular diameter was calculated as the average of the 2 measurements. Transrectal ultrasonography was performed on d -9 (GnRH1) and d 0 (GnRH2) to determine the diameter of the dominant follicle. The presence or absence of a class III follicle ( $>9$  mm; Moreira et al., 2000) was recorded at each ultrasound scan and used as an indicator of a potentially ovulatory follicle. Ovulatory follicles  $\leq 11$  mm were considered small dominant follicles, whereas follicles  $>11$  mm were considered large follicles. This cutoff was determined based on pregnancy rates in cows ovulating various follicle sizes reported previously in this herd (Perry et al., 2005). Ovarian ultrasound exams were performed daily from d -9 to 0 and on d 2 to characterize follicular waves and growth of dominant follicles and to confirm ovulation after GnRH1 and GnRH2, based on the disappearance of a dominant follicle and the formation of luteal tissue (after GnRH1). All ultrasound scans were recorded to video. Individual follicles were tracked beginning on d -5 to 0 to determine the long-term follicle growth rate. These days were chosen to monitor long-term growth because most cows had a follicle that could be tracked accurately during this period. In addition, ovaries were scanned daily beginning 9 (D18 group), 4 (D13 group), 2 (D9 and D5 groups), or 1 d (D2 group) before GnRH1 to characterize the dominant follicle before GnRH1 [i.e., follicle wave number and stage of growth (increasing, plateau, or regressing)]. Stage of growth at GnRH2 was assessed by fitting a polynomial curve to the follicle growth pattern of each cow. The first derivative of the polynomial was solved for zero to find the point on the

curve where the follicle was no longer growing ( $\pm 0.5$  d; plateau). The follicle was considered increasing in size before the time of plateau and was considered decreasing after the plateau.

### **Blood Collection and RIA**

Blood samples were collected via tail or jugular venipuncture into 10-mL vacuum tubes (Fisher Scientific, Pittsburgh, PA) daily from d  $-9$  to 21. After collection, the blood was incubated for 24 h at  $4^{\circ}\text{C}$ , followed by centrifugation at  $1,200 \times g$  for 25 min at  $4^{\circ}\text{C}$ . Serum was collected and stored at  $-20^{\circ}\text{C}$  until RIA. Serum concentrations of progesterone were measured in all samples using a Coat-a-Count RIA kit (Diagnostic Products Corporation, Los Angeles, CA; Kirby et al., 1997). Intra- and interassay CV were 2.9 and 9.8%, respectively. Sensitivity of the assay was 0.08 ng/mL. Two distinct luteal stages were characterized: 1) early luteolysis referred to cows that had undergone luteal regression before  $\text{PGF}_{2\alpha}$ , and 2) incomplete luteal regression referred to cows that had incomplete luteal regression after administration of  $\text{PGF}_{2\alpha}$ . Cows were determined to have undergone early luteolysis when serum concentrations of progesterone decreased below 1.0 ng/mL before  $\text{PGF}_{2\alpha}$ . Cows were determined to have incomplete luteal regression when serum concentrations of progesterone decreased after  $\text{PGF}_{2\alpha}$  but did not decrease below 1.0 ng/mL and then increased earlier than cows that had undergone complete luteal regression. Serum concentrations of estradiol- $17\beta$  were measured using RIA (Kirby et al., 1997) in samples collected from d  $-9$  to 0. Intra- and inter-assay CV were 13.1 and 17.6%, respectively. Sensitivity of the assay was 0.5 pg/mL.

### **Statistical Analyses**

One-way ANOVA with day as the independent variable was used to test differences among treatment groups in average follicular diameter and serum concentrations of estradiol at GnRH1 and GnRH2 (SAS Inst. Inc., Cary, NC). Differences in the proportion of cows ovulating among treatment groups at GnRH1 and GnRH2, undergoing premature luteolysis, and in estrus early were tested using GENMOD with SAS. Differences in average follicular diameter at GnRH1 and GnRH2 between cows that did or did not ovulate after GnRH1 were analyzed with the 2-sample *t*-test. The percentage of cows with a class III follicle from d  $-9$  through 0 was analyzed using the GENMOD procedure in SAS for repeated measures. Changes in serum concentrations of progesterone over time during the linear increase in progesterone (d 2 to 12 after GnRH2) were analyzed by ANOVA for repeated measures (PROC MIXED; Littell et al., 1998) between cows that ovulated small or large follicles after GnRH2. Because of unequal variances, the progesterone concentrations were log-transformed for the analysis; the actual concentrations were used

for graphical display. Differences in long-term follicle growth rate (d  $-5$  to 0) among treatment groups (day of the cycle), between cows that did or did not ovulate to GnRH1, and between cows with a small or large follicle at GnRH2 were analyzed by weighted ANOVA for repeated measures (PROC MIXED; Littell et al., 1998), in which time points were weighted based on the number of observations. A multiple regression with GLM in SAS was used to analyze the effect of ovulatory follicle diameter and display of estrus on serum concentrations of estradiol at the time of GnRH2. The correlations between ovulatory follicle diameter, follicle growth rate, and serum concentrations of estradiol were analyzed using the CORR procedure in SAS. Cows that underwent incomplete luteal regression after  $\text{PGF}_{2\alpha}$  were not included in the progesterone analysis after GnRH2 but were included in all other analyses.

## **RESULTS**

### **Ovulatory Response and Follicle Diameter**

The percentages of cows that ovulated to GnRH1 and GnRH2 were 50 and 76%, respectively. The percentage of cows ovulating at GnRH2 was greater when the largest follicle was increasing in size (32/34;  $P < 0.001$ ) or had reached a plateau in growth (10/11;  $P = 0.01$ ) compared with cows with a decreasing follicle diameter (4/7; cows that were in estrus before GnRH2 were not included in this analysis). The proportion of cows ovulating to GnRH2 was not different between cows with an increase in follicle growth and cows that had reached a plateau in follicle growth ( $P = 0.71$ ).

The size of the follicle at GnRH1 and the proportion that ovulated after GnRH1 was decreased in D2 cows ( $P < 0.003$  and  $0.005$ , respectively) compared with the remaining treatment groups. There was no difference in the proportion ovulating to GnRH1 among D5, D9, D13, or D18 cows ( $P > 0.2$ ; Table 1). Neither the proportion ovulating nor the size of the dominant follicle at GnRH2 was affected by cycle day at GnRH1 ( $P = 0.21$  and  $0.65$ , respectively; Table 1). Cycle day at GnRH1 affected the percentage of cows with a class III follicle between d  $-9$  and d 0 of the treatment period (cycle day  $\times$  time interaction  $P < 0.05$ ; Figure 1a); however, the percentage of cows with a class III follicle at GnRH2 was not affected by cycle day at the beginning of treatment ( $P > 0.2$ ; Figure 1a).

The range in ovulatory follicle diameter at GnRH2 was 7.7 to 18.2 mm, with 33% ovulating small ( $\leq 11$  mm) follicles. Ovulation to GnRH1 did not affect the proportion ovulating to GnRH2 or the size of the follicle at GnRH2 ( $P = 0.22$  and  $0.23$ , respectively; Table 2). Considering that cows on D2 of the cycle were unable to have a follicle capable of ovulating when administered GnRH1, a second analysis was performed to compare only cows capable of ovulating after GnRH1 (the D2 treatment group was removed). When cows on D2 of the estrous cycle were excluded, cows that ovulated

**Table 1.** Mean diameter (mm) of the largest follicle ( $\pm$ SEM) at the first and second GnRH injection (GnRH1 and GnRH2, respectively), number (%) of cows ovulating to GnRH1 and GnRH2, serum estradiol at GnRH2 (pg/mL, mean  $\pm$  SEM), number (%) of cows undergoing luteolysis before PGF<sub>2 $\alpha$</sub> , and number (%) of cows in estrus from GnRH1 to PGF<sub>2 $\alpha$</sub> <sup>1</sup>

Treatment group	GnRH1		GnRH2		Serum estradiol at GnRH2	Luteolysis before PGF <sub>2<math>\alpha</math></sub> <sup>2</sup>	In estrus before PGF <sub>2<math>\alpha</math></sub>
	Largest follicle diameter	Ovulating	Largest follicle diameter	Ovulating			
D2	6.5 $\pm$ 0.6 <sup>a</sup>	0/12 <sup>d</sup> (0)	11.7 $\pm$ 0.7	9/12 (75)	4.7 $\pm$ 1.0	0/12 <sup>a</sup> (0)	0/12 <sup>d</sup> (0)
D5	9.0 $\pm$ 0.3 <sup>b</sup>	9/12 <sup>e</sup> (75)	10.8 $\pm$ 0.5	9/12 (75)	3.2 $\pm$ 0.5	0/12 <sup>a</sup> (0)	0/12 <sup>d</sup> (0)
D9	11.5 $\pm$ 0.4 <sup>c</sup>	8/12 <sup>e</sup> (67)	10.8 $\pm$ 0.4	12/12 (100)	3.5 $\pm$ 0.5	0/12 <sup>a</sup> (0)	0/12 <sup>d</sup> (0)
D13	10.0 $\pm$ 0.4 <sup>bc</sup>	6/12 <sup>e</sup> (50)	11.4 $\pm$ 0.9	9/12 (75)	4.3 $\pm$ 0.6	11/12 <sup>b</sup> (92)	2/12 <sup>de</sup> (17)
D18	10.0 $\pm$ 0.5 <sup>bc</sup>	7/12 <sup>e</sup> (58)	9.8 $\pm$ 1.6	7/12 (58)	5.0 $\pm$ 1.0	5/12 <sup>c</sup> (42)	4/12 <sup>e</sup> (33)

<sup>a-c</sup>Means or percentages within a column having different superscripts were different ( $P < 0.01$ ).

<sup>d,e</sup>Means or percentages within a column having different superscripts were different ( $P < 0.05$ ).

<sup>1</sup>Treatment groups were based on the day of the estrous cycle at the beginning of the CO-Synch protocol [a first GnRH injection (GnRH1) was administered, followed 7 d later with an injection of PGF<sub>2 $\alpha$</sub> , and 48 h after PGF<sub>2 $\alpha$</sub> , a second injection of GnRH (GnRH2) was administered].

<sup>2</sup>Luteolysis was defined as the day when serum progesterone concentrations decreased below 1.0 ng/mL.

after GnRH1 had larger follicle diameters at GnRH2 than cows that did not ovulate ( $P = 0.04$ ; Table 2). Among cows that ovulated after GnRH2, the proportion of cows that ovulated a small follicle was not affected by ovulatory response to GnRH1 (7/25 and 10/21 of cows that did or did not ovulate to GnRH1 ovulated a small follicle at GnRH2, respectively;  $P = 0.17$ ). When D2 cows were removed from the analysis, there was still no difference in the proportion of cows that ovulated a small follicle with respect to ovulatory response at GnRH1 (7/25 and 6/12 of the cows that did or did not ovulate to GnRH1 ovulated a small follicle at GnRH2;  $P = 0.19$ ). Ovulation to GnRH1 affected the percentage of cows with a class III follicle throughout the synchronization period ( $P < 0.05$ ; Figure 1b). When D2 cows were excluded from the analysis, the percentage of cows with a class III follicle at GnRH2 that ovulated to GnRH1 was greater than the percentage of cows that did not ovulate to GnRH1 ( $P = 0.04$ ; Figure 1b).

### Luteolysis and Estrus

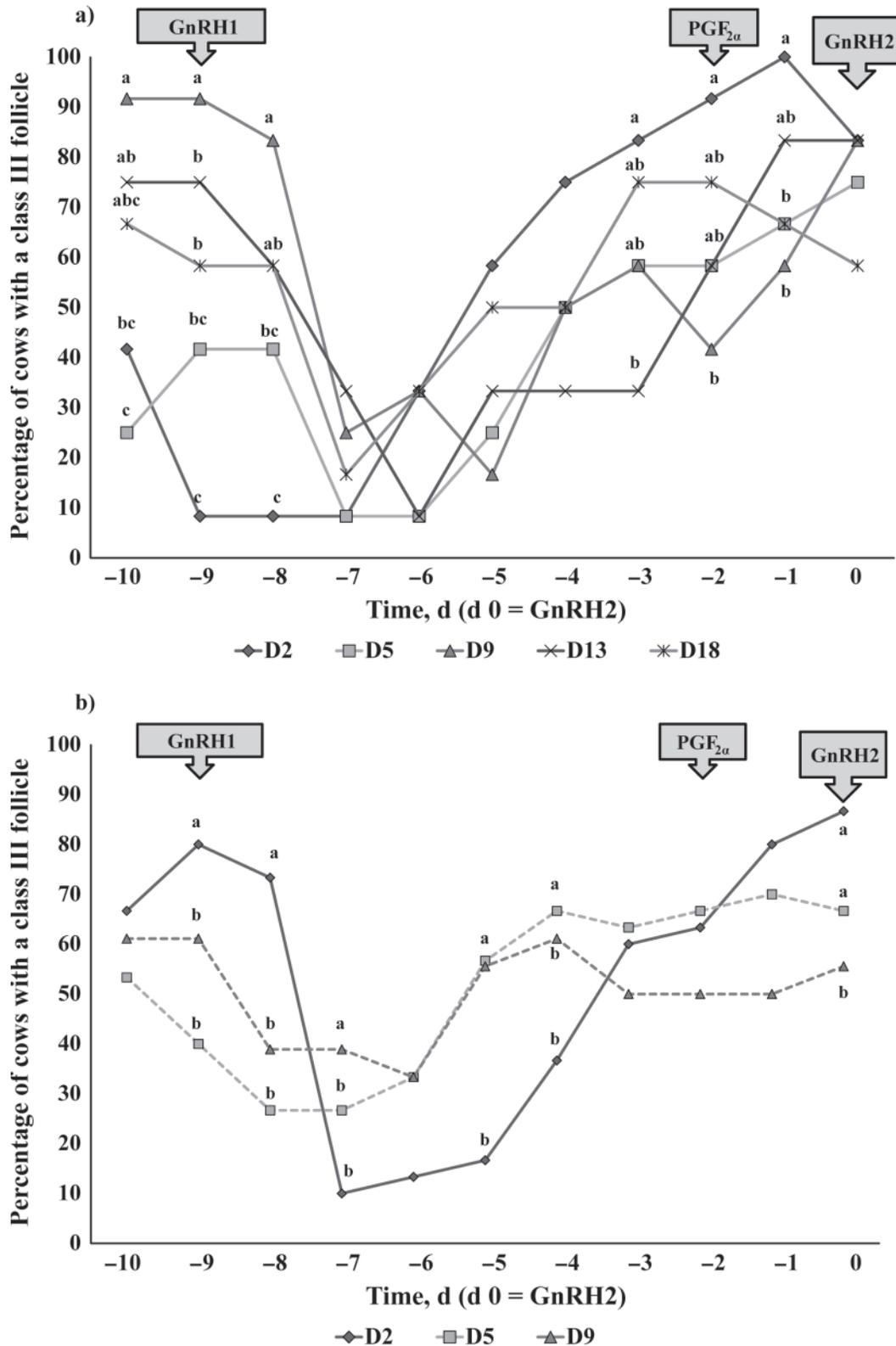
An increased proportion of cows in the later part of the estrous cycle at the beginning of the CO-Synch protocol underwent luteolysis before PGF<sub>2 $\alpha$</sub>  administration ( $P < 0.01$ ; Table 1). An increased proportion of cows in the D18 treatment group were in estrus before PGF<sub>2 $\alpha$</sub>  compared with cows in the D2, D5, or D9 treatment groups ( $P < 0.05$ ; Table 1). When all cows were included in the analysis, the proportion of cows undergoing luteolysis or in estrus before PGF<sub>2 $\alpha$</sub>  were not different between cows that ovulated or did not ovulate after GnRH1 ( $P = 0.24$  and  $P = 0.09$ , respectively; Table 2). When cows in the D2 treatment group were excluded from the analysis, fewer cows that ovulated to GnRH1 underwent luteolysis or displayed estrus before PGF<sub>2 $\alpha$</sub>  administration than cows that did not ovulate after GnRH1 ( $P < 0.001$  and  $P < 0.001$ , respectively; Table 2).

### Follicle Growth

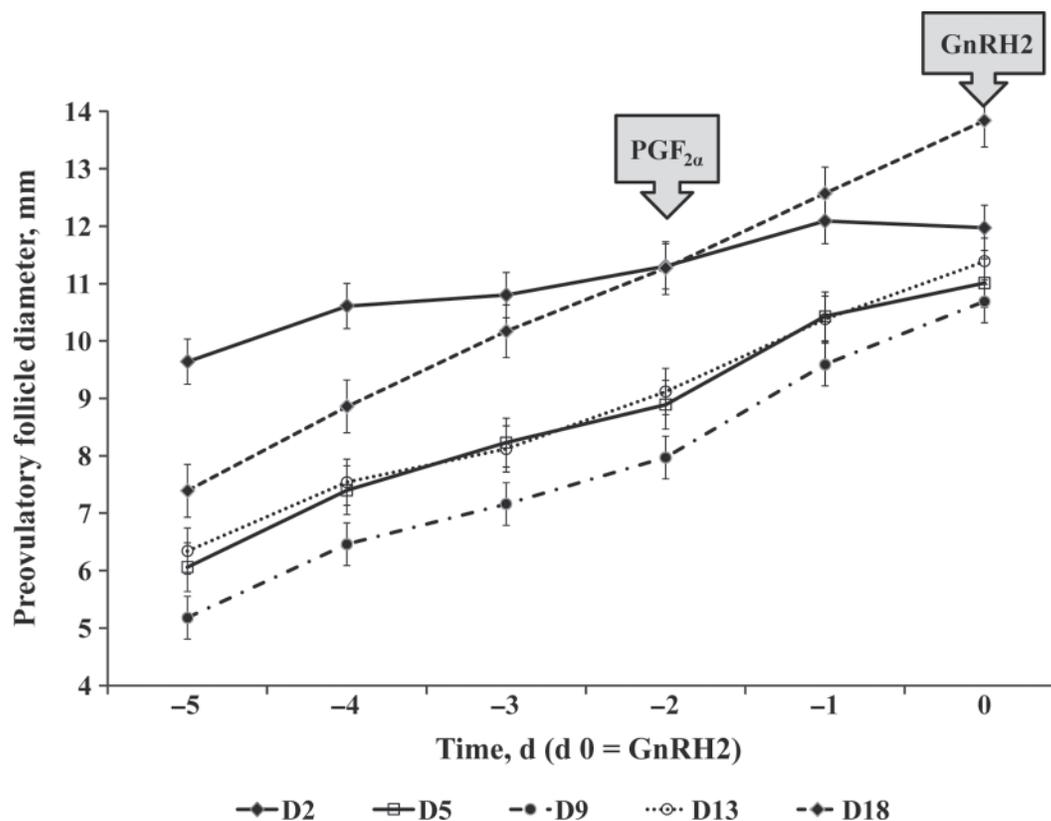
Only cows ovulating after GnRH2 were used to analyze follicle growth rate. Follicle growth from d -5 to 0 was not affected by day of the cycle at GnRH1 ( $P = 0.82$ ; Figure 2). When all cows were included in the analysis, there was no time  $\times$  ovulation to GnRH1 interaction ( $P = 0.75$ ; Figure 3). When D2 cows were removed from the analysis, there was also no time  $\times$  ovulation to GnRH1 interaction ( $P = 0.57$ ), but cows that ovulated after GnRH1 had a larger follicle by d -5 than cows that did not ovulate after GnRH1 ( $P = 0.05$ ; Figure 3). There was no interaction in long-term follicle growth between ovulation after GnRH1 and the size of the ovulatory follicle ( $P = 0.25$ ; data not shown). Mean follicle diameter  $\pm$  SEM for small follicles ( $\leq 11$  mm) was  $9.3 \pm 0.2$  mm, with a range of 7.7 to 10.7 mm, and mean follicle diameter  $\pm$  SEM for large follicles ( $> 11$  mm) was  $12.9 \pm 0.3$  mm, with a range of 11.1 to 18.2 mm. Follicle growth rate tended to be faster ( $P = 0.07$ ) from d -5 to 0 in cows ovulating large compared with small follicles (Figure 4), and follicle size was larger ( $P < 0.001$ ) from d -5 to 0 in these cows. Short-term (from d -2 to 0) growth rate and follicle diameter at GnRH2 were positively correlated ( $r = 0.467$ ;  $P < 0.0005$ ). Follicle growth rate was slower from d -2 to 0 in D2 cows than in D9 cows ( $0.339 \pm 0.14$  vs.  $1.29 \pm 0.08$  mm/d, respectively;  $P = 0.048$ ) but was similar among all other treatment groups ( $1.06 \pm 0.11$ ,  $1.13 \pm 0.12$ , and  $1.28 \pm 0.11$  mm/d for D5, D13, and D18 cows, respectively;  $P > 0.06$ ; Figure 2).

### Serum Concentrations of Progesterone and Estradiol

Serum concentrations of estradiol on the day of GnRH2 were not different among cows on different days of the cycle at GnRH1 ( $P = 0.33$ ; Table 1). Serum concentrations of estradiol on the day of GnRH2



**Figure 1.** Percentage of cows with a class III (>9 mm) follicle by cycle day at the first GnRH injection (GnRH1; panel a) and ovulatory response to GnRH1 (panel b) during the treatment period. Cows ( $n = 12$  per treatment group) were on d 2, 5, 9, 13, or 18 (referred to as D2, D5, D9, D13, and D18, respectively) of their estrous cycle at the beginning of the CO-Synch protocol [GnRH injection (GnRH1) followed 7 d later with  $\text{PGF}_{2\alpha}$ , and a second GnRH injection (GnRH2) 48 h after  $\text{PGF}_{2\alpha}$ ]. Cycle day at GnRH1 affected the percentage of cows with a class III follicle between d -9 and d 0 (time  $\times$  cycle day interaction;  $P < 0.05$ ); however, on d 0 (GnRH2) there was no effect of cycle day on the percentage of cows with a class III follicle ( $P > 0.2$ ). In panel b, the analysis compared cows that ovulated to GnRH1 (solid line with diamond points) with cows that did not ovulate to GnRH1 either with (hatched line with square points) or without (hatched line with triangle points) the D2 cows but did not compare the latter 2 groups. Treatment groups with different letters (a–e) had differences in the percentage of cows with a class III follicle ( $P < 0.05$ ).



**Figure 2.** Largest follicle diameter (y-axis) for the 5 d preceding the second GnRH injection (GnRH2; d 0) by treatment group [day of the cycle at the beginning of the CO-Synch protocol (GnRH injection followed 7 d later with  $\text{PGF}_{2\alpha}$ , and a second GnRH injection 48 h after  $\text{PGF}_{2\alpha}$ )]. D2, D5, D9, D13, and D18 refer to treatment groups on d 2, 5, 9, 13, and 18, respectively. Follicle growth was similar among cows on different days of the cycle at the beginning of the CO-Synch protocol ( $P = 0.83$  for the cycle day  $\times$  time interaction). Only cows that ovulated after GnRH2 are included.

were not different among cows that ovulated to GnRH1 compared with cows that did not ovulate after GnRH1 ( $P = 0.35$ ; Table 2). When the D2 cows were removed from the analysis, there was a tendency for cows that ovulated to GnRH1 to have increased serum concentrations of estradiol at GnRH2 compared with cows that did not ovulate to GnRH1 ( $P = 0.07$ ). In cows that ovulated to GnRH2, the serum concentrations of estradiol were positively correlated with size of the domi-

nant follicle at GnRH2 ( $r = 0.335$ ;  $P = 0.006$ ; Figure 5) independent of estrus.

The variance in the mean serum concentrations of progesterone was not equal over time, so the concentrations of progesterone were log-transformed for the analysis. Actual values are graphed in Figure 6. Ovulation of follicles  $\leq 11$  mm led to reduced concentrations of serum progesterone over time (Figure 6;  $P = 0.005$ ) compared with cows that ovulated follicles  $> 11$  mm in

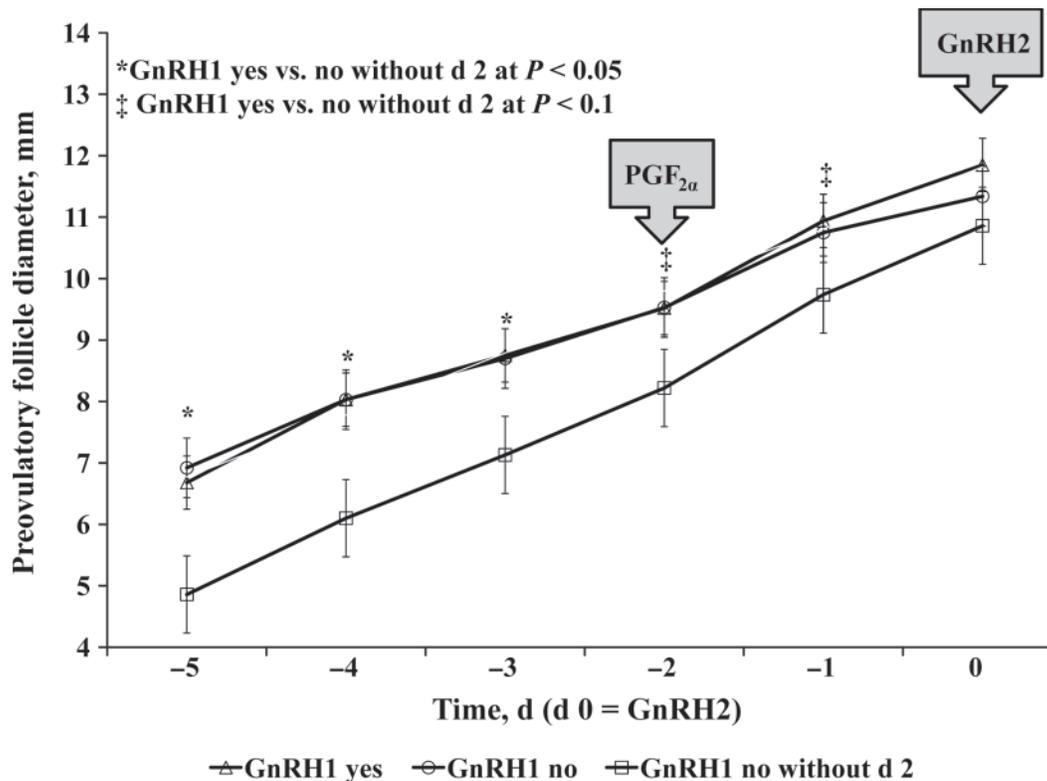
**Table 2.** Effect of ovulatory response at the first GnRH injection (GnRH1) in the CO-Synch protocol<sup>1</sup> on mean ( $\pm$  SEM) follicular diameter (mm) at GnRH1 and GnRH2, serum estradiol (pg/mL, mean  $\pm$  SEM) at GnRH2, number (%) of cows ovulating after GnRH2, number (%) of cows undergoing luteolysis before  $\text{PGF}_{2\alpha}$ , and number (%) of cows in estrus before  $\text{PGF}_{2\alpha}$ <sup>1</sup>

Ovulation, GnRH1	n	Follicle diameter, GnRH1	Follicle diameter, GnRH2	Estradiol at GnRH2	Ovulating after GnRH2	Luteolysis <sup>2</sup> before $\text{PGF}_{2\alpha}$	Estrus before $\text{PGF}_{2\alpha}$
Yes	30	10.6 $\pm$ 0.3	11.4 $\pm$ 0.5 <sup>a</sup>	4.46 $\pm$ 0.5 <sup>c</sup>	25/30 (83)	6/30 <sup>e</sup> (20)	1/30 <sup>e</sup> (3)
No with D2	30	8.4 $\pm$ 0.3	10.4 $\pm$ 0.7 <sup>a</sup>	3.85 $\pm$ 0.4 <sup>c</sup>	21/30 (70)	10/30 <sup>e</sup> (33)	5/30 <sup>e</sup> (17)
No without D2	18	9.7 $\pm$ 0.4	9.5 $\pm$ 0.8 <sup>b</sup>	3.25 $\pm$ 0.3 <sup>d</sup>	12/18 (67)	10/18 <sup>f</sup> (56)	5/18 <sup>f</sup> (28)

<sup>a-f</sup>Means within columns having different superscripts were different between cows that ovulated (yes) or cows that did not ovulate (either "no with" cows at D2 of the estrous cycle at GnRH1, or "no without" cows at D2 of the estrous cycle at GnRH1; <sup>a,b</sup> $P = 0.04$ , <sup>c,d</sup> $P < 0.1$ , <sup>e,f</sup> $P < 0.01$ ). D2 = d 2 of treatment.

<sup>1</sup>The CO-Synch protocol included a first injection of GnRH (GnRH1) followed 7 d later with an injection of  $\text{PGF}_{2\alpha}$ , and 48 h after  $\text{PGF}_{2\alpha}$ , a second injection of GnRH (GnRH2).

<sup>2</sup>Luteolysis was defined as the day the serum progesterone concentrations decreased below 1.0 ng/mL.



**Figure 3.** Growth of the preovulatory dominant follicle for 5 d preceding the second GnRH injection (GnRH2; d 0) between cows that did [first GnRH injection (GnRH1) yes;  $n = 25$ ] or did not (GnRH1 no;  $n = 21$ ) ovulate in response to GnRH1. Cows that ovulated to GnRH1 had a similar follicular growth pattern as cows that did not ovulate to GnRH1 when all treatment groups were included (GnRH1 yes and GnRH1 no;  $P = 0.75$ ). However, when the d 2 cows were excluded from the analysis (GnRH1 no without d 2,  $n = 12$ ), cows that ovulated to GnRH1 had a similar follicular growth rate from d -5 to 0 ( $P = 0.57$ ), but the size of the ovulatory follicle was larger at d -5 compared with cows that did not ovulate ( $P = 0.05$ ). Only cows that ovulated after GnRH2 are included.

diameter. Some cows from the D2 (6/12) and D5 (5/12) treatment groups underwent incomplete luteal regression after PGF<sub>2α</sub> (Figure 7) and were not included in the progesterone analysis. Of the cows that underwent incomplete luteolysis, 2/6 and 2/5 of the D2 and D5 cows, respectively, were considered to have ovulated after GnRH2 based on disappearance of a dominant follicle and formation of luteal tissue.

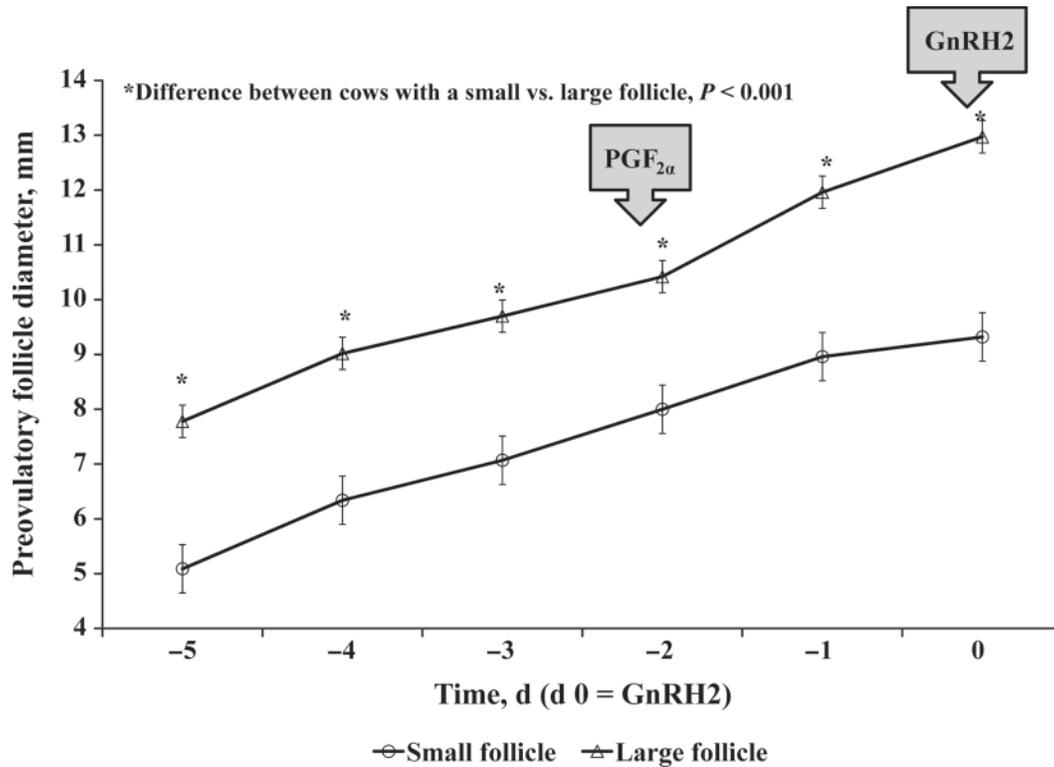
## DISCUSSION

In the present study, day of the estrous cycle at the beginning of the synchronization program did not affect follicle size or proportion ovulating at the second GnRH injection. In cows that were capable of ovulating to GnRH1 (i.e., excluding cows on D2 of the estrous cycle), cows that ovulated in response to GnRH1 had a larger follicle at GnRH2 than cows that did not ovulate to GnRH1. In anestrous beef cows, those that ovulated after the first GnRH injection also had larger follicles at the second GnRH injection than cows that did not ovulate after GnRH1 (Atkins et al., 2010). Vasconcelos et al. (1999) reported that day of the estrous cycle at the beginning of the Ovsynch program did not affect the percentage of dairy cows that ovulated at GnRH2, but cows in either the early (cycle d 1 to 4) or the later (cycle d 17 to 21) stage of the estrous cycle at

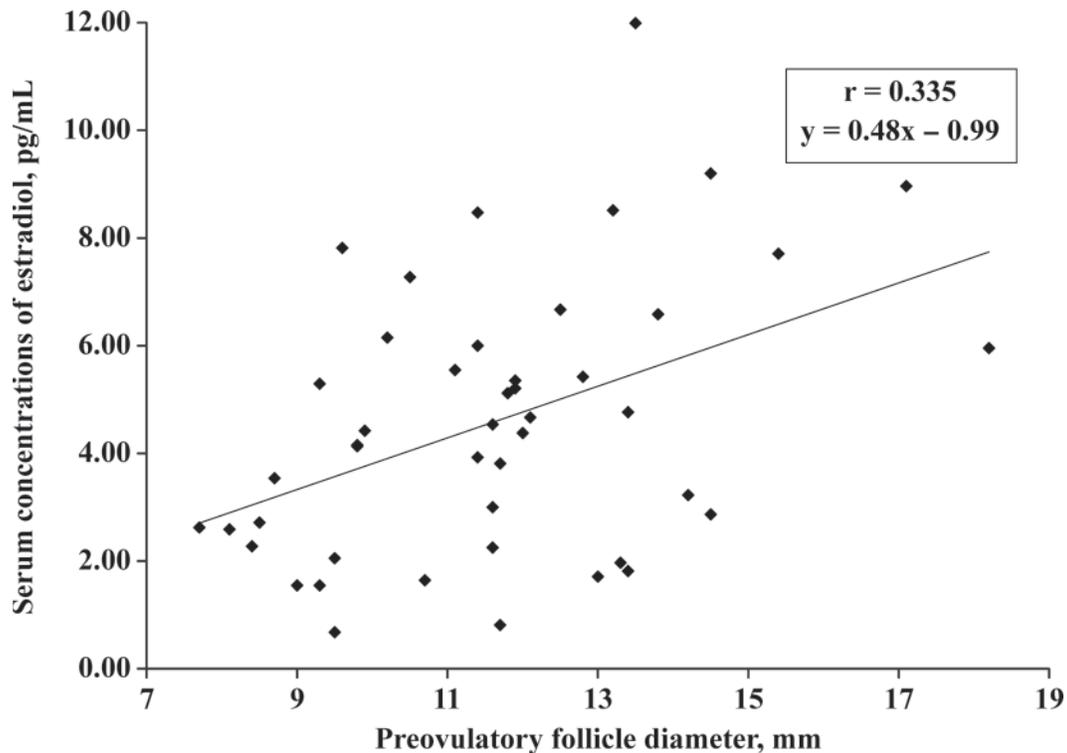
the beginning of synchronization had larger follicles at GnRH2 than cows in the middle of the estrous cycle. These results differ slightly from those of the current study, but the current study used defined days of the estrous cycle instead of a range in days.

Similar studies conducted in estrous cycling beef (Atkins et al., 2008) and dairy (Stevenson, 2008) heifers resulted in observations nearly opposite those of the current study. In beef heifers, ovulation to the first GnRH did not affect follicle size or ovulatory response at the second GnRH injection, but day of the estrous cycle at the beginning of the synchronization program did affect follicle size, percentage of heifers with a class III follicle at GnRH2, and ovulatory response after the second GnRH injection (Atkins et al., 2008). In dairy heifers, inclusion of the first GnRH injection did not improve response at the second GnRH or pregnancy rates (Stevenson, 2008), but day of the cycle did affect ovulation rate and follicle size at the second GnRH. The reason for the discrepancy between the heifer and cow responses is unknown.

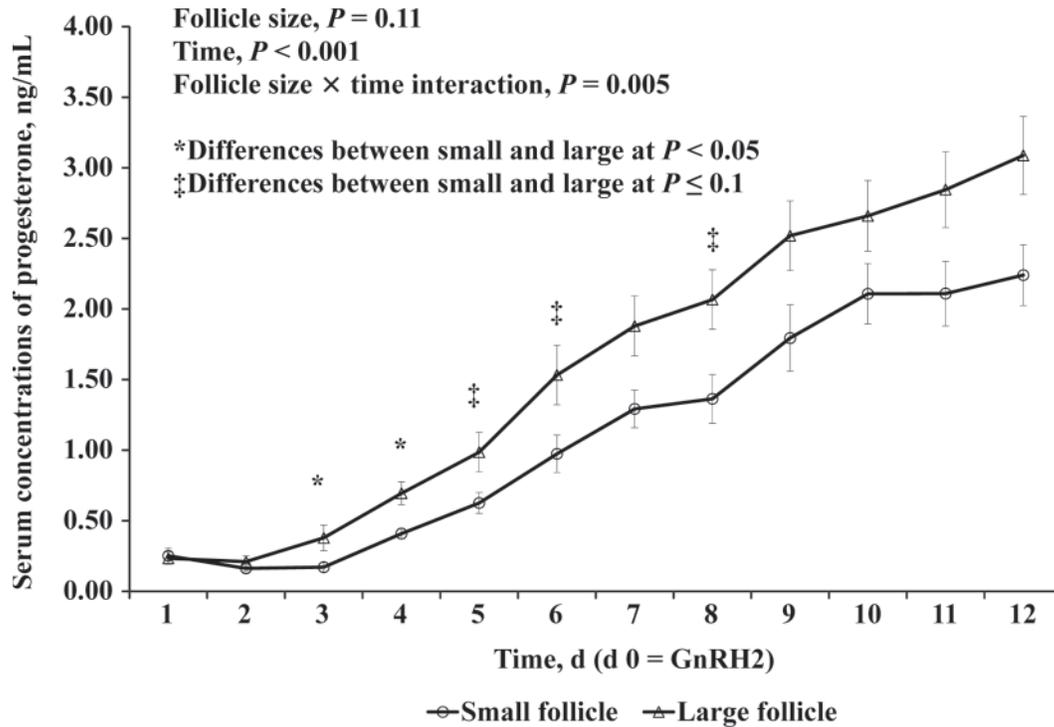
In the current study, nearly one-half of the cows in the early luteal phase at the beginning of the CO-Synch protocol underwent incomplete luteolysis after PGF<sub>2α</sub> (6/12 and 5/12 of the cows on D2 and D5 of the cycle at treatment initiation, respectively). Twagiramungu et al. (1994) reported that 4/18 cows treated with GnRH



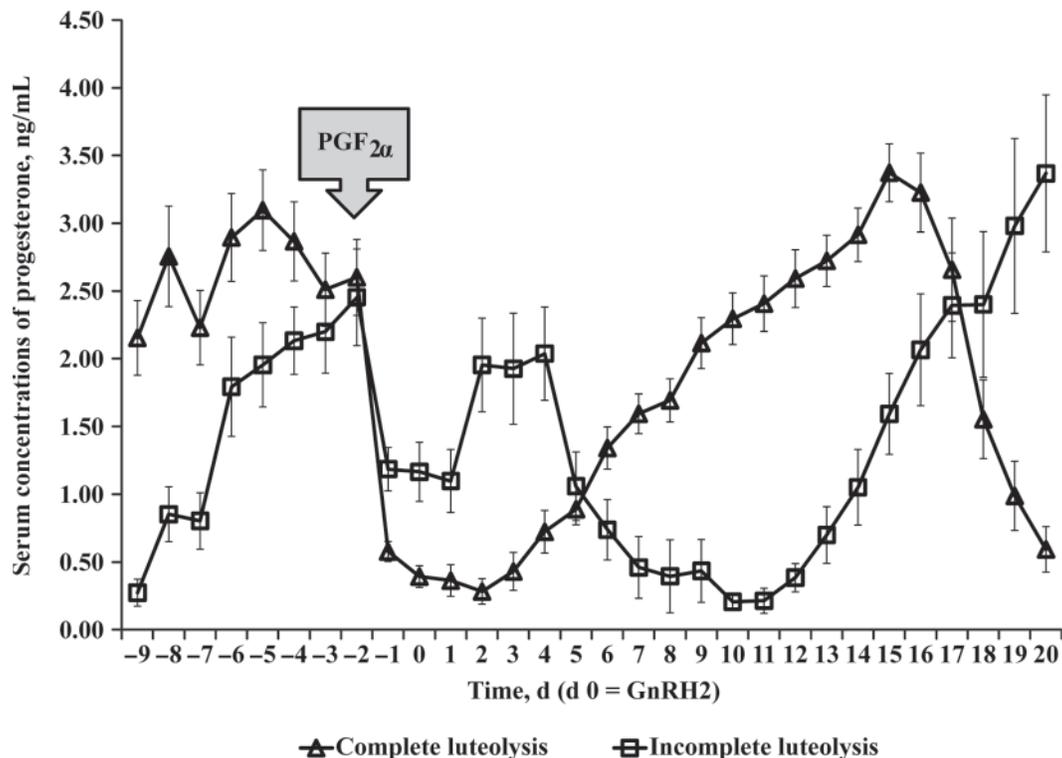
**Figure 4.** Growth of the preovulatory dominant follicle for 5 d preceding the second GnRH injection (GnRH2; d 0) among cows that ovulated a large ( $>11$  mm;  $n = 29$ ) or small ( $\leq 11$  mm;  $n = 17$ ) dominant follicle at GnRH2. Cows with a large follicle at GnRH2 had a faster rate of follicle growth leading up to GnRH2 than cows with a small dominant follicle at GnRH2 ( $P = 0.07$ ), and the large ovulatory follicles were larger at d -5 than the small ovulatory follicles ( $P < 0.001$ ). The complete regression equation is  $y = 9.8971 + 3.2812$  (follicle size group, where 0 = small and 1 = large)  $+ 0.4458$  (day)  $- 0.1310$  (day<sup>2</sup>). Only cows that ovulated after GnRH2 are included.



**Figure 5.** Scatter plot of serum concentration of estradiol (pg/mL) and diameter of the preovulatory follicle at the second GnRH injection (GnRH2). Serum concentration of estradiol was positively correlated ( $r = 0.335$ ;  $P = 0.006$ ) with diameter of the dominant follicle at GnRH2. Only cows that ovulated after GnRH2 are included ( $n = 46$ ).



**Figure 6.** Mean serum concentrations of progesterone from d 1 to 12 after the second GnRH injection (GnRH2; d 0) in cows that ovulated small follicles (<11 mm; n = 14; circles) or large follicles ( $\geq 11$  mm; n = 24; triangles). Because of unequal variance over time, the mean concentrations of progesterone were log-transformed for the analysis, but the nontransformed data are presented in this graph. Cows that ovulated large follicles had a greater increase in serum concentrations of progesterone than cows that ovulated small follicles ( $P = 0.005$ ). Only cows that ovulated after GnRH2 and underwent complete luteal regression are included.



**Figure 7.** Mean serum concentrations of progesterone (ng/mL; error bars = SEM) during the treatment period and the subsequent estrous cycle. Some cows on treatment d 2 (6/12) and treatment d 5 (5/12) of the cycle at the beginning of the CO-Synch protocol [GnRH injection (GnRH1) followed 7 d later with PGF<sub>2α</sub>, and a second GnRH injection (GnRH2) 48 h after PGF<sub>2α</sub>] underwent incomplete luteolysis after PGF<sub>2α</sub> administration (open squares), whereas the remaining cows (from all treatment groups) had complete luteal regression (open triangles) based on serum concentrations of progesterone.

followed 7 d later with PGF<sub>2α</sub> underwent incomplete luteolysis. Similarly, Burke et al. (1996) reported that 8% of lactating dairy cows had incomplete luteal regression following PGF<sub>2α</sub> administered 7 d after GnRH. It is interesting that in the present study, only the cows in the early part of the estrous cycle had incomplete luteal regression, and all the cows on D9, D13, or D18 underwent complete luteolysis. Future experiments to confirm whether day of the cycle may contribute to the ability to undergo complete luteolysis after PGF<sub>2α</sub> administration could be important to improving the success of estrous synchronization protocols.

Fortune et al. (1988) reported the growth rate of the dominant follicle in the first, second, and third follicular wave to be 1.6, 1.1, and 1.7 mm/d, respectively, in Holstein heifers. In the present study, follicle growth rate (0.89 mm/d; 5 d before GnRH2) was a little slower compared with the expected 1 to 2 mm/d. Follicle growth rate increased slightly after PGF<sub>2α</sub> administration (0.99 mm/d) compared with the long-term growth rate. The increase in follicle growth rate after PGF<sub>2α</sub> may be due to the loss of negative feedback from progesterone on LH (Beck et al., 1976); therefore, an increase in LH pulse frequency could drive follicle growth and estradiol production (Fortune, 1994). Although follicle growth rate from d -5 to 0 was similar between cows that did or did not ovulate after GnRH1, cows that ovulated after GnRH1 had a larger follicle by d -5 than cows that did not ovulate after GnRH1. The larger follicular diameter by d -5 may have been due to the synchronized growth of a follicular wave in cows ovulating to GnRH1.

The reason for the reduced fertility associated with ovulation of a smaller dominant follicle may be related to the altered hormone profile in the cows after ovulation of an immature follicle. Britt and Holt (1988) described the 5 critical periods of changing hormone profiles that are important to fertility in lactating cows: 1) the luteal phase during the estrous cycle before insemination, 2) the period from the onset of luteolysis to estrus, 3) the preovulatory period, 4) the period from ovulation until progesterone increases, and 4) the luteal phase after insemination. Cows that are induced to ovulate a small dominant follicle may have altered endocrine profiles at several of these important periods, which could affect fertility. In the current study, cows that ovulated a small dominant follicle had reduced serum concentrations of estradiol during the preovulatory period and reduced serum concentrations of progesterone in the subsequent luteal phase. Cows that were induced to ovulate a small dominant follicle had reduced serum concentrations of estradiol at the time of ovulation compared with cows that ovulated large or small follicles spontaneously (Vasconcelos et al., 2001; Busch et al., 2008). Our results are supported by several others who reported reduced serum concentrations of progesterone in the subsequent luteal phase after induced ovulation of small (Perry et al., 2005; Atkins et al., 2008; Busch et al., 2008) or immature (Burke et al.,

2001; Mussard et al., 2007) dominant follicles. Luteal secretion of progesterone is vital to embryo survival. Inadequate luteal function may impair interferon- $\tau$  production (Mann and Lamming, 2001) or uterine secretions important to embryo development (Garrett et al., 1988). The mechanism by which induced ovulation of small dominant follicles affects the establishment and maintenance of pregnancy is unclear, but may be related to an inadequate uterine environment supported by altered endocrine profiles of these cows, or to poor oocyte quality, gamete transport, or oviductal environment. Current studies are underway using reciprocal embryo transfer experiments to attempt to resolve this question.

In summary, day of the estrous cycle at initiation of the CO-Synch protocol did not affect ovulatory follicle size or ovulatory response at induced ovulation for timed AI. Ovulatory response to GnRH1 also did not affect ovulatory response to GnRH2 and affected ovulatory size only when cows on d 2 of their estrous cycle at the start of synchronization (which did not have a follicle capable of ovulating at GnRH1) were removed from the analysis. Follicle growth rate leading up to GnRH2 tended to be faster in cows that ovulated a large follicle compared with cows that ovulated a small follicle. Ovulatory follicle diameter was positively correlated with serum concentrations of estradiol at GnRH2, and cows that ovulated small follicles had a smaller increase in serum concentrations of progesterone 12 d after GnRH.

## LITERATURE CITED

- Atkins, J. A., D. C. Busch, J. F. Bader, D. H. Keisler, D. J. Patterson, M. C. Lucy, and M. F. Smith. 2008. Gonadotropin-releasing hormone-induced ovulation and luteinizing hormone release in beef heifers: Effect of day of the cycle. *J. Anim. Sci.* 86:83-93.
- Atkins, J. A., M. F. Smith, K. J. Wells, and T. W. Geary. 2010. Factors affecting preovulatory follicle diameter and ovulation rate following gonadotropin-releasing hormone in postpartum beef cows. Part II: Anestrous cows. *J. Anim. Sci.* 88:2311-2320. 10.2527/jas.2009-2532
- Beck, T. W., V. G. Smith, B. E. Seguin, and E. M. Convey. 1976. Bovine serum LH, GH, and prolactin following chronic implantation of ovarian steroids and subsequent ovariectomy. *J. Anim. Sci.* 42:461-468.
- Britt, J. H., and L. C. Holt. 1988. Endocrinological screening of embryo donors and embryo transfer recipients: A review of research with cattle. *Theriogenology* 29:189-202.
- Burke, C. R., M. L. Mussard, D. E. Grum, and M. L. Day. 2001. Effects of maturity of the potential ovulatory follicle on induction of oestrus and ovulation in cattle with oestradiol benzoate. *Anim. Reprod. Sci.* 66:161-174.
- Burke, J. M., R. L. De la Sota, C. A. Risco, C. R. Staples, E. J.-P. Schmitt, and W. W. Thatcher. 1996. Evaluation of timed insemination using a gonadotropin-releasing hormone agonist in lactating dairy cows. *J. Dairy Sci.* 79:1385-1393.
- Busch, D. C., J. A. Atkins, J. F. Bader, D. J. Schafer, D. J. Patterson, T. W. Geary, and M. F. Smith. 2008. Effect of ovulatory follicle size and expression of estrus on progesterone secretion in beef cows. *J. Anim. Sci.* 86:553-563.
- Fortune, J. E. 1994. Ovarian follicular growth and development in mammals. *Biol. Reprod.* 50:225-232.

- Fortune, J. E., J. Sirois, and S. M. Quirk. 1988. The growth and differentiation of ovarian follicles during the bovine estrous cycle. *Theriogenology* 29:95–109.
- Garrett, J. E., R. D. Geisert, M. T. Zavy, and G. L. Morgan. 1988. Evidence for maternal regulation of early conceptus growth and development in beef cattle. *J. Reprod. Fertil.* 84:437–446.
- Geary, T. W., and J. C. Whittier. 1998. Effects of a timed insemination following synchronization of ovulation using the Ovsynch or CO-Synch protocol in beef cows. *Prof. Anim. Sci.* 14:217–220.
- Ginther, O. J., M. A. Beg, D. R. Bergfelt, F. X. Donadeu, and K. Kot. 2001. Follicle selection in monovular species. *Biol. Reprod.* 65:638–647.
- Kirby, C. J., M. F. Smith, D. H. Keisler, and M. C. Lucy. 1997. Follicular function in lactating dairy cows treated with sustained-release bovine somatotropin. *J. Dairy Sci.* 80:273–285.
- Lamb, G. C., J. S. Stevenson, D. J. Kesler, H. A. Garverick, D. R. Brown, and B. E. Salfen. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F<sub>2α</sub> for ovulation control in postpartum suckled beef cows. *J. Anim. Sci.* 79:2253–2259.
- Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* 76:1216–1231.
- Mann, G. E., and G. E. Lamming. 2001. Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. *Reproduction* 121:175–180.
- Moreira, F., R. L. de la Sota, T. Diaz, and W. W. Thatcher. 2000. Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. *J. Anim. Sci.* 78:1568–1576.
- Mussard, M. L., C. R. Burke, E. J. Behlke, C. L. Gasser, and M. L. Day. 2007. Influence of premature induction of a luteinizing hormone surge with gonadotropin-releasing hormone on ovulation, luteal function, and fertility in cattle. *J. Anim. Sci.* 85:937–943.
- Perry, G. A., M. F. Smith, M. C. Lucy, J. A. Green, T. E. Parks, M. D. MacNeil, A. J. Roberts, and T. W. Geary. 2005. Relationship between follicle size at insemination and pregnancy success. *Proc. Natl. Acad. Sci. USA* 102:5268–5273.
- Stevenson, J. S. 2008. Progesterone, follicular and estrual responses to progesterone-based estrus and ovulation synchronization protocols at five stages of the estrous cycle. *J. Dairy Sci.* 91:4640–4650.
- Twagiramungu, H., L. A. Guilbault, J. G. Proulx, and J. J. Dufour. 1994. Influence of corpus luteum and induced ovulation on ovarian follicular dynamics in postpartum cyclic cows treated with buserelin and cloprostenol. *J. Anim. Sci.* 72:1796–1805.
- Vasconcelos, J. L., R. Sartori, H. N. Oliveira, J. G. Guenther, and M. C. Wiltbank. 2001. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. *Theriogenology* 56:307–314.
- Vasconcelos, J. L., R. W. Silcox, G. J. M. Rosa, J. R. Pursley, and M. C. Wiltbank. 1999. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. *Theriogenology* 52:1067–1078.

**Related Articles**

A related article has been published:  
<http://jas.fass.org/cgi/content/abstract/88/7/2311>

**References**

This article cites 22 articles, 14 of which you can access for free at:  
<http://jas.fass.org/cgi/content/full/88/7/2300#BIBL>