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

Part II: Anestrous cows¹

J. A. Atkins,* M. F. Smith,* K. J. Wells,*² and T. W. Geary†³

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

ABSTRACT: There is large variation in dominant follicle diameter at the time of GnRH-induced ovulation in the CO-Synch protocol [a first GnRH injection on d -9 (GnRH1), followed by PGF_{2α} on d -2, and a second GnRH injection (GnRH2) with timed AI on d 0], and the reason for the presence of small dominant follicles at GnRH2 is not known. Our hypothesis was that ovulatory response to GnRH1 and progesterone exposure [controlled intravaginal drug-releasing insert (CIDR; EAZI-Breed, Pfizer Animal Health, New York, NY)] would affect ovulatory follicle size at GnRH2 in anestrous cows. This study used a 2 × 2 factorial arrangement of treatments in which anestrous suckled beef cows (n = 55) either ovulated (Ov1+) or failed to ovulate (Ov1-) after GnRH1 and either received (CIDR+) or did not receive (CIDR-) a 7-d CIDR treatment (from GnRH1 to PGF_{2α}), resulting in the following treatment groups: Ov1+CIDR+, Ov1-CIDR+, Ov1+CIDR-, and Ov1-CIDR- (n = 9, 17, 11, and 18, respectively). The Ov1+ cows had larger follicles at GnRH2 (12.3 vs. 11.0 mm; *P* = 0.04), a decreased proportion of small follicles within cows that ovulated to GnRH2 (2/16 vs. 14/23; *P* = 0.003), and a similar growth rate of the ovulatory follicle from d -5 to 0 (d 0

= GnRH2; 1.1 ± 0.06 vs. 1.1 ± 0.07 mm/d; *P* = 0.99) compared with Ov1- cows. Administration of a CIDR had no effect on follicle diameter at GnRH2 (11.8 vs. 11.2 mm; *P* = 0.3), proportion of small ovulatory follicles at GnRH2 (7/19 vs. 9/20; *P* = 0.6), and follicular growth rate from d -5 to 0 (d 0 = GnRH2; 1.2 ± 0.07 vs. 1.1 ± 0.07 mm/d; *P* = 0.76). Administration of a CIDR, but not ovulation to GnRH1, increased follicle growth from d -2 to 0 (d 0 = GnRH2; *P* = 0.03 and 0.9, respectively). Large follicles (>11 mm) had a similar growth rate from d -5 to 0 (d 0 = GnRH2; *P* = 0.44) compared with small follicles (1.1 ± 0.07 vs. 1.2 ± 0.07 mm/d), but the large ovulatory follicles were larger at d -5 compared with small ovulatory follicles (*P* < 0.001). Follicle diameter was positively correlated with serum concentrations of estradiol at GnRH2 (*r* = 0.622; *P* < 0.0001). In summary, ovulation to GnRH1, but not CIDR administration, resulted in increased dominant follicle diameter at GnRH2 in anestrous suckled beef cows. Large follicles were already larger 5 d before GnRH2 but grew at a rate similar to small follicles; follicle size was positively correlated with serum concentrations of estradiol at the time of GnRH-induced ovulation.

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

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INTRODUCTION

Multiple reports indicated that cows induced to ovulate small dominant follicles have reduced pregnancy

rates (Lamb et al., 2001; Vasconcelos et al., 2001; Perry et al., 2005). There is large variation in the diameter of the largest follicle (Lamb et al., 2001; Perry et al., 2005) at the time of GnRH-induced ovulation

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

³Corresponding author: Tom.Geary@ars.usda.gov

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Table 1. Mean (\pm SEM) days postpartum (DPP), age, and BCS for each treatment group

| Treatment ¹ | n | DPP, d | Age, yr | BCS ² |
|------------------------|----|--------------|---------------|------------------|
| Ov1+CIDR+ | 9 | 42 \pm 2.3 | 3.4 \pm 0.8 | 4.3 \pm 0.1 |
| Ov1+CIDR- | 11 | 42 \pm 1.8 | 3.9 \pm 0.8 | 4.6 \pm 0.1 |
| Ov1-CIDR+ | 17 | 44 \pm 1.5 | 2.9 \pm 0.4 | 4.2 \pm 0.1 |
| Ov1-CIDR- | 18 | 44 \pm 1.7 | 2.7 \pm 0.3 | 4.3 \pm 0.1 |

¹Ovulation to the first GnRH injection (GnRH1; Ov1+) or failure to ovulate (Ov1-) and presence (CIDR+) or absence (CIDR-) of a controlled internal drug-releasing insert (CIDR; EAZI-Breed, Pfizer Animal Health, New York, NY). There were no treatment differences ($P > 0.6$).

²BCS was based on a 1 to 9 scale, where 1 = emaciated and 9 = obese.

in the CO-Synch protocol [GnRH on d 9 (**GnRH1**), PGF_{2 α} on d 2, and GnRH on d 0 (**GnRH2**); Geary and Whittier, 1998] and other timed AI protocols [CO-Synch plus controlled intravaginal drug-releasing insert (**CIDR**; Lamb et al., 2001)]. Additionally, cows that were induced to ovulate a small dominant follicle had an increased occurrence of late embryonic or fetal mortality (Perry et al., 2005). The reason for the presence of small dominant follicles at GnRH2 is unknown, but may be due to failure to control the initiation of a new follicular wave before GnRH2. This lack of control of the follicle wave could result in more variation in the age and size of the follicle at GnRH2. Alternatively, varied follicle growth rates leading up to GnRH2 could contribute to variation in preovulatory follicle diameter.

In anestrous cows, the pulse frequency and mean concentrations of circulating LH were less than those in cycling cows, which could reduce the rate of dominant follicle growth (reviewed by Yavas and Walton, 2000). Administration of a progestin increased the frequency of LH pulses in postpartum beef cows (Williams et al., 1983; Garcia-Winder et al., 1986). Addition of exogenous progesterone to the CO-Synch protocol may affect follicle growth rate and the preovulatory follicle diameter at GnRH2.

We hypothesized that failure to initiate a new follicular wave after GnRH1 and absence of progesterone would increase the occurrence of small preovulatory follicles at the GnRH-induced ovulation. The objectives of the study were to determine the effect of ovulation after GnRH1 and exogenous progesterone treatment on the size and growth rate of the largest follicle at GnRH2 in anestrous 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

Anestrous suckled beef cows (n = 55) at the Fort Keogh Livestock and Range Research Laboratory were administered the CO-Synch protocol, with the excep-

tion that no cows were bred. Anestrous status was based on serum concentrations of progesterone 10 d before the beginning of treatment and the absence of a corpus luteum (**CL**) at treatment initiation. Approximately one-half of the cows were administered exogenous progesterone (EAZI-Breed CIDR containing 1.38 g of progesterone, Pfizer Animal Health, New York, NY; n = 26) from GnRH1 to PGF_{2 α} , resulting in a 2 \times 2 factorial arrangement of treatments based on ovulation (**OV1+**) or failure to ovulate (**Ov1-**) to GnRH1 and the presence (**CIDR+**) or absence (**CIDR-**) of a CIDR (Ov1+CIDR+, Ov1-CIDR+, Ov1+CIDR-, Ov1-CIDR-; n = 9, 17, 11, and 18, respectively). There were no differences in average days postpartum, age, or BCS (1 to 9, where 1 = emaciated and 9 = obese) among the treatment groups (Table 1). Estrus was detected visually twice daily from d -9 to 20 (d 0 = GnRH2) and was aided with the use of Estrus Alert (Western Point Inc., Apple Valley, MN) estrous detection patches.

Transrectal Ultrasonography

Ovarian structures were monitored using an Aloka 500V ultrasound instrument with a 7.5-MHz transducer (Aloka, Wallingford, CT). Follicles ≥ 5 mm in diameter and the presence of a CL were recorded. Follicle diameter was measured at the widest point and at a right angle to the first measurement, and the average of these measurements was recorded as the follicle diameter. Transrectal ultrasonography was performed on d -9 (GnRH1) and d 0 (GnRH2) to determine the diameter of the ovulatory follicle. The presence of a class III follicle (>9 mm; Moreira et al., 2000) was recorded at each ultrasound exam and used as an indicator of a follicle that might ovulate. Ovulatory follicles ≤ 11 mm were considered small dominant follicles and follicles >11 mm were considered large follicles based on previous research defining the optimal follicle diameter for pregnancy in this herd (Perry et al., 2005). Ovulation after GnRH1 and GnRH2 was determined on d -7 and 2, respectively, and was based on the disappearance of a dominant follicle and, in some cases, formation of new luteal tissue. Daily ultrasound exams from d -9 to 0 were performed and recorded to monitor the growth of dominant follicles during the treatment period. The

long-term (d -5 to 0) follicle growth pattern required backtracking specific follicles using the recorded ovarian sonograms. Most cows had an individual follicle from a single follicular wave that was tracked beginning on d -5, so the growth of the ovulatory follicle was calculated from d -5 to 0. A polynomial equation was fit to the follicle growth curve, and the first derivative of the polynomial equation was determined for each cow. The first derivative was solved for zero to determine the day the follicle had reached a plateau in growth (± 0.5 d). Follicles were considered to be increasing in size before the plateau and decreasing in size after the plateau.

Blood Collection and RIA

Blood samples were collected daily from d -9 to 20 by tail or jugular venipuncture into 10-mL vacuum tubes (Fisher Scientific, Pittsburgh, PA). After collection, the blood was stored for 24 h at 4°C, followed by centrifugation at $1,200 \times g$ for 25 min at 4°C. Serum was harvested and stored at -20°C until RIA. Serum concentrations of progesterone were measured in all samples by using a Coat-a-Count RIA kit (Diagnostic Products Corporation, Los Angeles, CA; Kirby et al., 1997). The intra- and interassay CV for the progesterone RIA were 3.7 and 8.4%, respectively. The sensitivity of the assay was 0.08 ng/mL. Serum concentrations of estradiol-17 β were measured by using RIA (Kirby et al., 1997) in samples collected from d -9 to 0. The intra- and interassay CV were 9.5 and 18.8%, respectively. The sensitivity of the assay was 0.5 pg/mL.

Resumption of the Estrous Cycle

Based on the serum concentrations of progesterone from d 0 to 20 and estrous data, cows were classified as cycling or anestrous (serum concentrations of progesterone remained below 1.0 ng/mL for the duration of the experiment). The cycling cows were further separated into cows with a normal estrous cycle (≥ 16 d) or cows with a short estrous cycle (< 16 d; Rantala et al., 2009).

Statistical Analyses

Throughout the analyses, the main effects of CIDR administration and ovulation to GnRH1 were analyzed, followed by interaction of the main effects. The proportions of cows that ovulated to GnRH, had a small ovulatory follicle, and resumed estrous cycling were analyzed using the GENMOD procedure (SAS Inst. Inc., Cary, NC). The percentage of cows with a class III follicle during the treatment period was analyzed using the GENMOD procedure for repeated measures over time. The main effect of CIDR administration and ovulation to GnRH1 on the average follicle diameter at GnRH1 and GnRH2, short-term follicle growth rates (from d -2 to 0), and serum concentrations of estradiol was analyzed by one-way ANOVA using the 2-sample

t-test in SAS, whereas the interaction of the treatments was analyzed using a GLM with treatment as the independent variable. Long-term follicle growth (from d -5 to 0) was analyzed by a weighted ANOVA for repeated measures over time (PROC MIXED; Littell et al., 1998), in which time points were weighted according to the number of observations recorded at the time points. The correlation between concentrations of estradiol and ovulatory follicle diameter was analyzed with the CORR procedure in SAS. Additionally, a multiple regression model was used to analyze estrus and follicle size as the independent variables and serum concentrations of estradiol as the dependent variable (PROC GLM in SAS). The increase in serum concentrations of progesterone after GnRH2 between cows that ovulated a small vs. large follicle was analyzed using the MIXED procedure for repeated measures (Littell et al., 1998).

RESULTS

Ovulatory Response and Follicle Diameter

Ovulatory responses to GnRH1 and GnRH2 were 36 and 71%, respectively. A larger proportion ($P < 0.05$) of cows with a follicle that was increasing in diameter at GnRH2 ovulated after GnRH2 than cows with a follicle that had reached a plateau in growth or cows with a decreasing follicle diameter at GnRH2 (20/22, 1/9, and 12/23 for cows with an increase, plateau, or decrease in follicle growth, respectively). Neither ovulation to GnRH1 nor CIDR administration affected the proportion of cows ovulating to GnRH2 ($P = 0.27$ and 0.73, respectively; Table 2).

The range in ovulatory follicle diameter was 8.6 to 16.1 mm, with 41% of the follicles ≤ 11 mm. Ovulation to GnRH1, but not CIDR treatment, resulted in a larger follicle at GnRH2 ($P = 0.04$ and 0.3, respectively; Table 2; for each treatment group, see Table 3), and there was more variation ($P < 0.05$) in ovulatory follicle diameter at GnRH2 in the Ov1- cows compared with the Ov1+ cows (variance was 4.0 vs. 2.9, respectively). When only cows that ovulated to GnRH2 were analyzed, the Ov1+ treatment group had a decreased proportion of cows that ovulated small follicles in response to GnRH2 compared with the Ov1- treatment group (Ov1+ had 2/16 small ovulatory follicles and Ov1- had 14/23 small ovulatory follicles; $P = 0.0025$). Administration of CIDR did not affect the proportion of small ovulatory follicles at GnRH2 (CIDR+ had 7/19 small ovulatory follicles and CIDR- had 9/20 small ovulatory follicles; $P = 0.61$) or the variation in ovulatory follicle diameter ($P > 0.10$).

The percentage of cows with a class III follicle (> 9 mm) was affected by treatment day ($P = 0.0005$). Ovulation to GnRH1 and CIDR treatment did not affect the percentage of cows with a class III follicle during the treatment period (Figure 1a and 1b; $P = 0.9$) nor was there an interaction of these treatments (Figure 1c; $P = 0.21$).

Table 2. Main treatment effects of ovulation to the first GnRH injection (GnRH1; Ov1+) or failure to ovulate (Ov1-) and presence (CIDR+) or absence (CIDR-) of a controlled internal drug-releasing (CIDR) insert¹ on the proportion (%) ovulating after the second GnRH injection (GnRH2), size of the largest follicle (mm; mean \pm SEM), and serum concentrations of estradiol (pg/mL; mean \pm SEM) at GnRH2

| Treatment | n | Ovulation after GnRH2 | Dominant follicle diameter | Estradiol |
|-----------|----|-----------------------|-----------------------------|----------------------------|
| GnRH1 | | | | |
| Ov1+ | 20 | 16/20 (80) | 12.3 \pm 0.5 ^a | 3.4 \pm 0.5 ^a |
| Ov1- | 35 | 23/35 (66) | 11.0 \pm 0.3 ^b | 2.3 \pm 0.3 ^b |
| CIDR | | | | |
| CIDR+ | 26 | 19/26 (73) | 11.8 \pm 0.4 | 2.8 \pm 0.4 |
| CIDR- | 29 | 20/29 (69) | 11.2 \pm 0.4 | 2.5 \pm 0.3 |

^{a,b}Means within a column with different superscripts differed between treatments ($P < 0.05$).

¹EAZI-Breed (Pfizer Animal Health, New York, NY).

Follicle Growth

The average long-term (d -5 to 0) and short-term (d -2 to 0) follicle growth rate across all cows was 1.1 and 0.89 mm/d, respectively. Among cows that ovulated to GnRH2, Ov1+ cows had similar follicle growth from d -5 to 0 compared with Ov1- cows (1.1 \pm 0.06 and 1.1 \pm 0.06 mm/d, respectively; Figure 2; $P = 0.99$) and from d -2 to 0 (0.77 \pm 0.17 and 0.70 \pm 0.15 mm/d, respectively; $P = 0.9$). The ovulatory follicle was already larger on d -5 in Ov1+ cows compared with Ov1- cows (Figure 2; $P = 0.003$). Among cows that ovulated to GnRH2, CIDR+ cows had a faster growth rate from d -2 to 0 (0.97 \pm 0.15 and 0.50 \pm 0.15 mm/d, respectively; $P = 0.03$) compared with CIDR- cows, but the growth rate was similar from d -5 to 0 (1.2 \pm 0.07 and 1.1 mm/d \pm 0.07, respectively; $P = 0.99$; Figure 2). There was no interaction between CIDR administration and ovulation after GnRH1 in either long-term ($P = 0.97$) or short-term ($P = 0.17$) follicle growth. Follicle growth was similar ($P = 0.44$) from d -5 to 0 in cows ovulating large (>11 mm) compared with small follicles at GnRH2 (1.1 \pm 0.07 and 1.2 \pm 0.07 mm/d, respectively; Figure 3), but cows that ovulated a large follicle at GnRH2 already had a larger follicle at d -5 compared with cows with a small ovulatory follicle (Figure 3; $P < 0.001$). The short-term follicle growth rate

was positively correlated with the size of the follicle at GnRH2 ($r = 0.44$ and $P = 0.005$).

Serum Concentrations of Estradiol and Estrus

Ovulation to GnRH1, but not CIDR treatment, resulted in increased serum concentrations of estradiol at GnRH2 ($P = 0.004$ and 0.59 , respectively; Table 2). The serum concentrations of estradiol were positively correlated with size of the dominant follicle at GnRH2 ($r = 0.62$; $P < 0.0001$; Figure 4). Only 7 cows were in estrus at the time of GnRH2, and both estrus and follicle diameter had a significant positive relationship with serum concentrations of estradiol on the day of GnRH2 ($P < 0.0001$ and $P = 0.003$, respectively).

Resumption of Cyclicity

The proportion of cows that resumed cycling after GnRH2 was similar between Ov1+ and Ov1- cows (16/20 and 20/35, respectively; $P = 0.11$). Similarly, there was no effect of CIDR administration on the proportion of cows that resumed cycling ($P = 0.33$), nor was there an interaction among treatments ($P > 0.10$; Table 4). Of the cows that resumed cycling, there was a treatment interaction in the proportion of cows with

Table 3. Proportion (%) ovulating, size of the largest follicle (mm; mean \pm SEM), and serum concentrations of estradiol (pg/mL; mean \pm SEM) at the second GnRH injection (GnRH2) in each individual treatment group

| Treatment ¹ | n | Ovulation after GnRH2 | Dominant follicle diameter | Estradiol |
|------------------------|----|-----------------------|----------------------------|-----------------------------|
| Ov1+CIDR+ | 9 | 7/9 (78) | 12.3 \pm 0.9 | 3.9 \pm 0.8 ^a |
| Ov1+CIDR- | 11 | 9/11 (82) | 12.3 \pm 0.7 | 3.6 \pm 0.6 ^{ab} |
| Ov1-CIDR+ | 17 | 12/17 (71) | 11.6 \pm 0.4 | 2.4 \pm 0.4 ^{ab} |
| Ov1-CIDR- | 18 | 11/18 (61) | 10.5 \pm 0.5 | 2.1 \pm 0.3 ^b |

^{a,b}Means within a column with different superscripts differ ($P = 0.07$).

¹Ovulation (Ov1+) to the first GnRH injection (GnRH1) or failure to ovulate (Ov1-) and presence (CIDR+) or absence (CIDR-) of a controlled internal drug-releasing insert (CIDR; EAZI-Breed, Pfizer Animal Health, New York, NY).

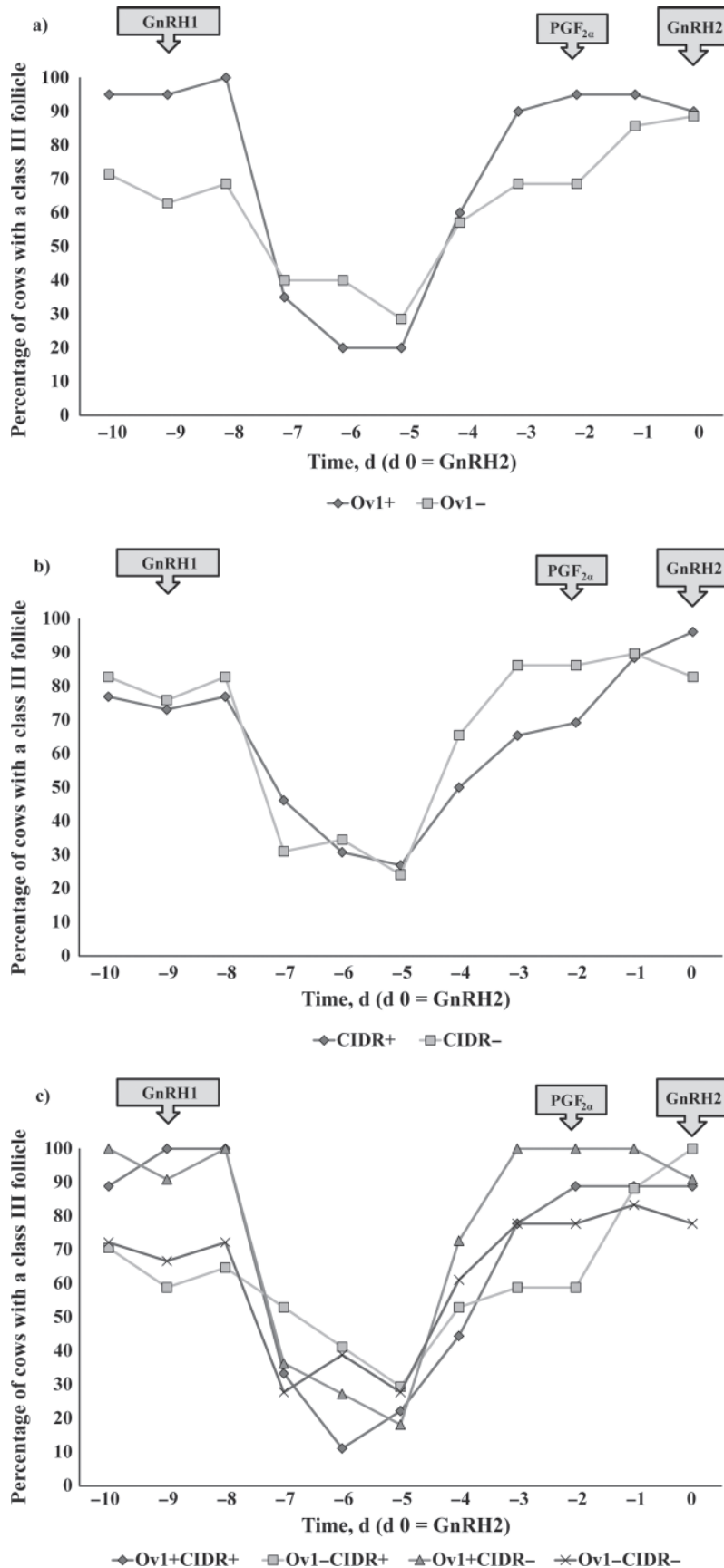


Figure 1. Percentage of cows with a class III follicle (>9 mm) during the treatment period. All cows were administered a first GnRH injection (GnRH1) on d -9, $\text{PGF}_{2\alpha}$ on d -2, and a second GnRH injection (GnRH2) on d 0. There was no difference in percentage of cows with a class III follicle between cows that did (Ov1+) or did not ovulate (Ov1-) after GnRH1 (panel a; $P = 0.09$) during the treatment period. Similarly, the percentage of cows with a class III follicle during the treatment period did not differ between those administered a controlled internal drug-releasing insert (CIDR; EAZI-Breed, Pfizer Animal Health, New York, NY; CIDR+) or not (CIDR-; panel b; $P = 0.9$). There was no interaction between ovulatory response and CIDR administration on the percentage of cows with a class III follicle over time (panel c; $P = 0.21$).

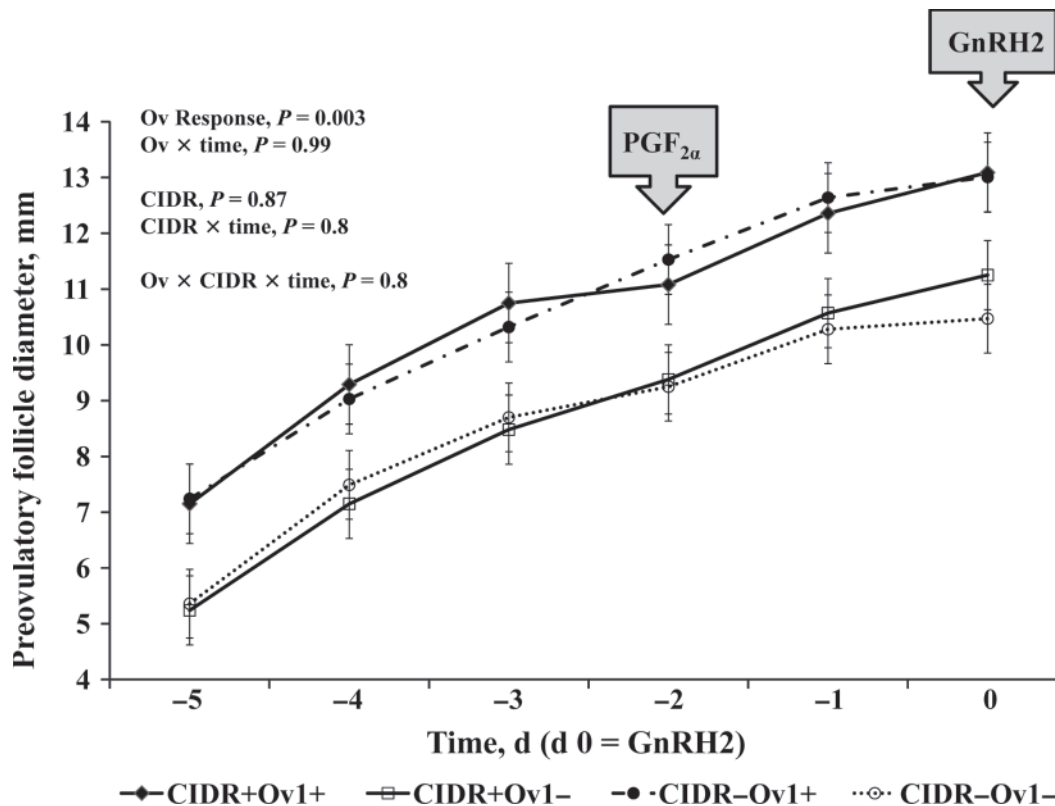


Figure 2. The preovulatory follicle diameter leading up to the second GnRH injection (GnRH2) by treatment. All cows were administered the first GnRH injection (GnRH1) on d -9, PGF_{2α} on d -2, and GnRH2 on d 0, and only cows that ovulated after GnRH2 were included. Cows that ovulated after GnRH1 (Ov1+; n = 16) had a similar follicular growth rate from d -5 to 0 ($P = 0.99$) and from d -2 to 0 ($P = 0.9$) compared with cows that did not ovulate after GnRH1 (Ov1-; n = 23). The ovulatory follicle was larger at d -5 in Ov1+ cows compared with Ov1- cows ($P = 0.003$). Cows that received a controlled internal drug-releasing insert (CIDR; EAZI-Breed, Pfizer Animal Health, New York, NY; CIDR+; n = 19) had a similar follicular growth rate from d -5 to 0 ($P = 0.76$) compared with cows that did not receive a CIDR (CIDR-; n = 20) but had a faster follicular growth rate from d -2 to 0 ($P = 0.03$).

a short cycle. Cows in the Ov1-CIDR- group had a smaller proportion of normal estrous cycle lengths (more cows with a short cycle) than Ov1+ and CIDR+ cows ($P < 0.05$; Table 4). None of the cows classified as having normal estrous cycles had estrous cycles shorter than 19 d. Cows that ovulated a large follicle after GnRH2 had greater ($P < 0.05$) serum concentrations of progesterone by d 4 after GnRH2 than cows that ovulated a small follicle (Figure 5).

DISCUSSION

In the current study, there was large variation in ovulatory follicle size at GnRH2. Perry et al. (2005) reported a range in ovulatory follicle diameter of 9 to 20 mm at the time of GnRH2 and AI, and Atkins et al. (2010) reported a range of 7.7 to 18.2 mm in cycling beef cows, with 43% of the follicles that ovulated being ≤ 11 mm. Ovulatory follicle size and physiological maturity of the follicle are implicated in contributing to the establishment and maintenance of pregnancy in beef (Lamb et al., 2001; Perry et al., 2005; Mussard et al., 2007) and dairy cattle (Vasconcelos et al., 2001). Lamb et al. (2001) reported that beef cows ovulating a follicle < 12 mm in diameter had reduced pregnancy rates compared with cows that ovulated follicles ≥ 12 mm. Perry et al.

(2005) also reported that cows induced to ovulate small dominant follicles had reduced serum concentrations of progesterone after ovulation, reduced initial pregnancy rates (d 28 after AI), and more late embryonic or fetal loss by d 60 to 68 of gestation.

Cows that ovulated after GnRH1 did have larger follicles at GnRH2 compared with cows that did not ovulate after GnRH1. In both cycling (Atkins et al., 2010) and anestrous cows (current study), cows that ovulated after GnRH1 had a larger follicle by d -5 than cows that did not ovulate after GnRH1, and these cows continued to have a larger follicle up to GnRH2. The presence of the larger follicle at d -5 to 0 may indicate an improved control of the dominant follicle at GnRH2 in cows that ovulated after GnRH1 compared with cows that did not ovulate after GnRH1. Cows that failed to ovulate at GnRH1 likely had either a follicle that was too young (small) to ovulate in response to the GnRH-induced LH surge (Sartori et al., 2001) or an atretic follicle. Considering the former scenario, these cows would be expected to have a dominant follicle that was more advanced in age at GnRH2 that may have become atretic or undergone follicular turnover before the GnRH2 injection compared with cows that ovulated to GnRH1. However, another possibility is that the dominant follicle of that follicular wave had not yet reached

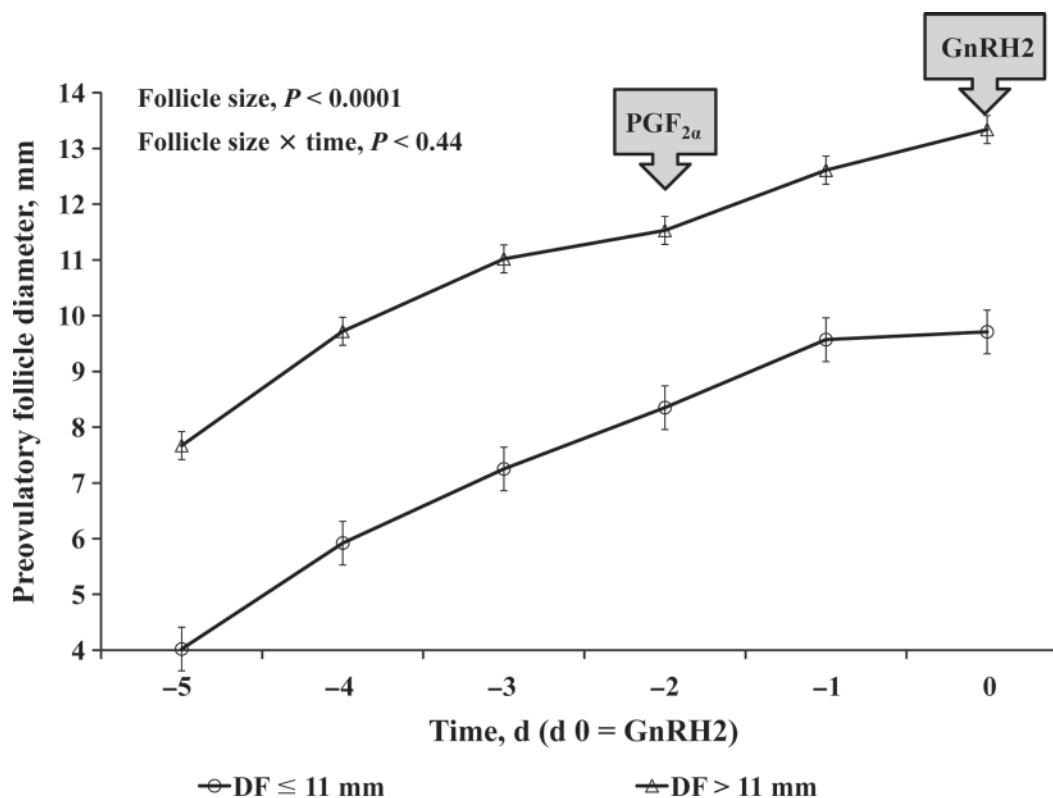


Figure 3. The preovulatory follicle diameter leading up to the second GnRH injection (GnRH2) by ovulatory follicle size. All cows were administered a first GnRH injection (GnRH1) on d -9, PGF_{2 α} on d -2, and GnRH2 on d 0, and only cows that ovulated after GnRH2 were included. Cows with a large ovulatory follicle (>11 mm; $n = 22$) had a similar follicular growth rate from d -5 to 0 compared with cows that ovulated a small follicle (≤ 11 mm; $P = 0.44$; $n = 17$), but the follicle was already larger by d -5 in cows that ovulated a large follicle compared with cows that ovulated a small follicle ($P < 0.001$). DF = dominant follicle.

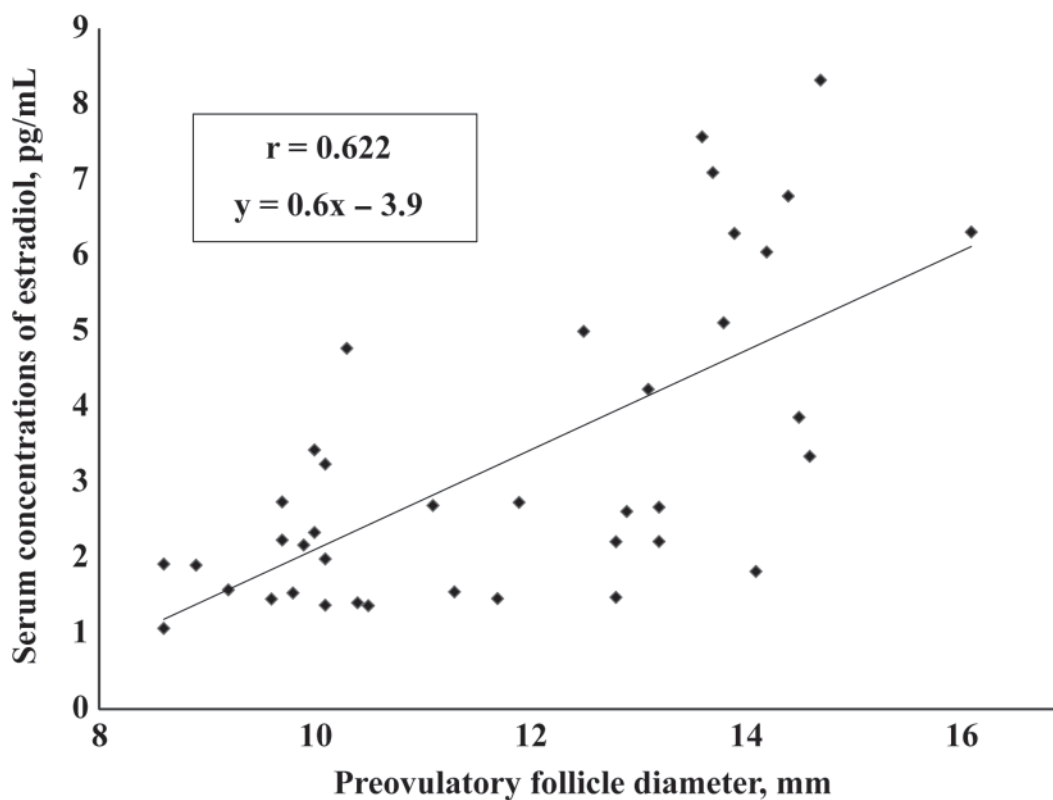


Figure 4. The ovulatory follicle diameter and serum concentrations of estradiol at the second GnRH injection (GnRH2) were positively correlated ($r = 0.622$, $P < 0.0001$). Only cows that ovulated after GnRH2 were included in the analysis ($n = 39$).

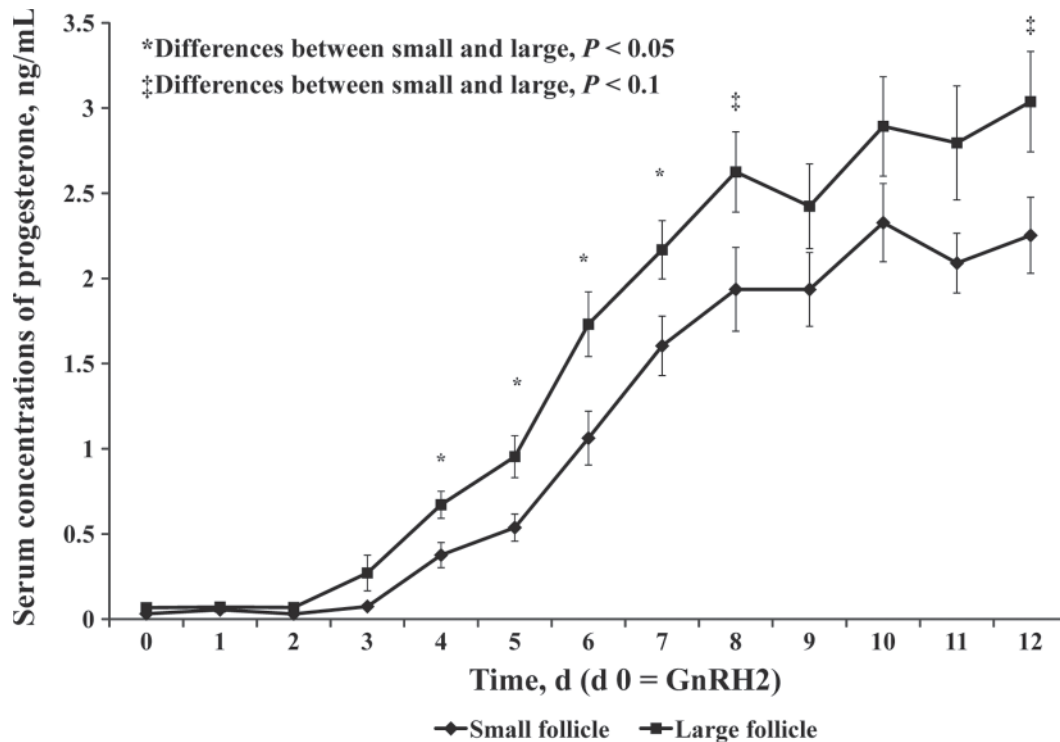


Figure 5. Serum concentration of progesterone for cows that ovulated a small (≤ 11 mm; $n = 8$) or large follicle (> 11 mm; $n = 18$) after the second GnRH injection (GnRH2). There was no follicle size group \times time interaction ($P = 0.2$), but differences between small and large follicle groups on specific days are indicated on the graph. Only cows that ovulated after GnRH2 and that had a normal luteal cycle were included.

a critical size or maturation such that it contained LH receptors and was capable of ovulating. Cows with an atretic follicle at GnRH1 would likely begin a new follicular wave around the same time as cows that ovulated to GnRH1 and may have a similarly synchronized follicular wave.

Table 4. Proportion (%) of cows that were cycling after the second GnRH injection (GnRH2) and, of those cycling, proportion (%) having a normal-length estrous cycle per treatment group

| Treatment ¹ | n | Cycling ² | Normal cycle ³ |
|------------------------|----|----------------------|---------------------------|
| Ov1+CIDR+ | 9 | 7/9 (78) | 7/7 (100) ^a |
| Ov1+CIDR- | 11 | 9/11 (82) | 8/9 (89) ^a |
| Ov1-CIDR+ | 17 | 12/17 (71) | 10/12 (89) ^a |
| Ov1-CIDR- | 18 | 8/18 (47) | 1/8 (12) ^b |

^{a,b}Means within a column with different superscripts differ among treatments ($P < 0.05$).

¹Ovulation after the first GnRH injection (GnRH1; Ov1+) or failure to ovulate (Ov1-) and the presence (CIDR+) or absence (CIDR-) of a controlled internal drug-releasing insert (CIDR; EAZI-Breed, Pfizer Animal Health, New York, NY).

²Resumption of cyclicity was based on the pattern of serum concentrations of progesterone after GnRH2. All cows that had either a short luteal cycle followed by a second increase in progesterone or a regular-length estrous cycle were considered to be cycling. Cows that continued to have serum concentrations of progesterone less than 1.0 ng/mL for the duration of the experiment were considered anestrous.

³Of the cows that resumed estrous cycling, cows that had a short increase in serum concentrations of progesterone (< 16 d) were considered to have a short cycle. Cows that had increased concentrations of progesterone consistent with a regular estrous cycle (18 to 24 d) were considered to be normal cycling.

Follicular growth rate in the current study was similar to the expected 1 to 2 mm/d (Fortune et al., 1988; Murphy et al., 1990) and to growth rates reported from the same herd in cyclic beef cows (0.89 mm/d; Atkins et al., 2010). There was no difference in long-term follicle growth (5 d leading up to GnRH2) between cows with a CIDR insert and cows without the CIDR insert. Garcia-Winder et al. (1986) reported that anestrous cows administered a progestogen supplement (norgestomet) had an increase in LH pulse frequency 5 d after the beginning of treatment compared with cows without the supplement. It is possible that CIDR administration did not affect the long-term growth of the ovulatory follicle because of timing of the increase in LH frequency. Progestogen supplementation increased follicular weight, concentrations of estradiol in follicular fluid, and LH receptors in the thecal and granulosa cells in postpartum beef cows compared with cows without supplementation (Inskeep et al., 1988), which may explain the increased short-term follicle growth rate in CIDR+ compared with CIDR- cows.

Many reports indicate that progesterone supplementation can induce cyclicity in anestrous cows (Smith et al., 1987; Twagiramungu et al., 1995; Lucy et al., 2001). In the current study, neither CIDR administration nor ovulation to GnRH1 increased the number of cycling cows. This was unexpected, because both CIDR administration (Lucy et al., 2001) and GnRH-induced ovulation (Twagiramungu et al., 1995) were able to induce noncycling heifers and cows to cycle; we may not have had a sufficient number of cows in the cur-

rent study to detect such a difference. The CL that forms after the first ovulation generally has a shortened lifespan compared with subsequent CL (Berardinelli et al., 1979; LaVoie et al., 1981) in suckled beef cows. Among cows that did return to estrous cycling, those that either received a CIDR or ovulated after GnRH1 (and therefore had a CL secreting progesterone) had a reduced incidence of short luteal phases compared with Ov1–CIDR– cows. This shortened luteal phase occurs because of an earlier release of PGF_{2α} from the uterus (Copelin et al., 1989). Exposure to progesterone followed by estrogen is needed to correct the timing of the PGF_{2α} release from the uterus (Cooper et al., 1991; Kieborz-Loos et al., 2003). Administration of exogenous progesterone to anestrous cows primes the uterus for appropriate timing of the PGF_{2α} release, resulting in a normal CL lifespan and estrous cycle length. Similar to that of estrous cycling cows (Atkins et al., 2010), ovulation of a large follicle compared with ovulation of a small follicle after GnRH2 resulted in increased progesterone concentrations during the subsequent estrous cycle.

In the current study, circulating concentrations of estradiol at GnRH2 increased as the ovulatory follicle diameter increased, which has been reported previously in dairy cows (Vasconcelos et al., 2001), beef heifers (Atkins et al., 2008), and cyclic beef cows (Atkins et al., 2010). Ireland et al. (1979) reported increased follicular fluid concentrations of estradiol in larger follicles compared with small follicles. The increase in serum concentrations of estradiol may be indicative of a more physiologically mature follicle. Mussard et al. (2007) also reported reduced serum concentrations of progesterone and pregnancy rates after GnRH-induced ovulation of immature follicles. Perry et al. (2005) reported increased pregnancy rates among cows with elevated serum estradiol concentrations at GnRH2 that were induced to ovulate large or small follicles.

Concentrations of estradiol around the time of ovulation play a significant role in several events leading to the establishment of pregnancy, including sperm transport (Hawk, 1983), uterine pH (Perry and Perry, 2008a,b), follicular cell maturation (McNatty, 1979), and improved oviductal (Buhi, 2002) and uterine environment (Miller and Moore, 1976; Ing and Tornesi, 1997). Taken together, this evidence indicates that adequate serum concentrations of estradiol around ovulation can improve pregnancy by optimizing gamete transport, luteal function, and oviductal and uterine environment. Ovulation of small follicles may limit serum concentrations of estradiol and affect subsequent events associated with fertilization and pregnancy success.

In summary, anestrous cows that ovulated after GnRH1 had larger follicles, and fewer of those cows ovulated a small follicle compared with cows that did not ovulate after GnRH1. Administration of a CIDR did not affect ovulatory follicle diameter. The long-term follicular growth rate was independent of the ovu-

latory response after GnRH1, CIDR administration, or size of the ovulatory follicle at GnRH2 in anestrous cows. Administration of a CIDR did increase the short-term follicle growth rate. Ovulatory follicle diameter was positively correlated with serum concentrations of estradiol on the day of GnRH2 in anestrous cows. We conclude that in anestrous beef cows, ovulation to GnRH1 increased the size of the ovulatory follicle at GnRH2 and decreased the proportion of cows ovulating a small follicle at GnRH2. Thus, increasing the proportion of cows ovulating to GnRH1 would likely increase the fertility of anestrous cows ovulating at GnRH2.

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