

# Effect of ovulatory follicle size and expression of estrus on progesterone secretion in beef cows

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**ABSTRACT:** Induced ovulation of small dominant follicles (SF, < 12 mm; CO-Synch protocol) in postpartum beef cows resulted in formation of corpora lutea (CL) that exhibited a delayed rise in progesterone (P4) compared with CL from large dominant follicles (LF, > 12 mm). Experiment 1 characterized P4 concentrations from ovulation to subsequent estrus among GnRH-induced or spontaneously ovulated SF (< 11 mm) or LF (> 12 mm) to determine if P4 secretion by CL formed from GnRH-induced SF remains lower postovulation in nonlactating beef cows. Nonlactating beef cows were induced to ovulate 48 h after PGF<sub>2α</sub> (CO-Synch; GnRH on d -9, PGF<sub>2α</sub> on d -2, and GnRH on d 0) or exhibited estrus and spontaneously ovulated after PGF<sub>2α</sub>. Follicle size was measured at the second GnRH in cows induced to ovulate or ~3 h after the onset of estrus for cows that ovulated spontaneously. Cows were classified into 1 of 4 groups: 1) GnRH-induced ovulation-SF (< 11 mm; Ind-SF; n = 9); 2) GnRH-induced ovulation-LF (> 12 mm; Ind-LF; n = 16); 3) spontaneous ovulation-SF (< 11 mm; Spon-SF; n = 8); 4) spontaneous ovulation-LF (> 12 mm; Spon-LF; n = 22). Serum concentrations of P4 from d 3 to 15 were reduced in the Ind-SF compared with the Ind-LF ( $P = 0.05$ ), Spon-SF ( $P = 0.07$ ), and Spon-

LF ( $P = 0.03$ ). Experiment 2 characterized P4 concentrations (0 to 60 d postAI) among GnRH-induced or spontaneously ovulated SF (< 12 mm) or LF (> 13 mm) to determine if P4 secretion by CL formed from GnRH-induced SF remained lower during early gestation. Ovulation was induced with GnRH 48 h after PGF<sub>2α</sub> (CO-Synch) or occurred spontaneously, and ovulatory follicle size was measured at AI. Lactating cows were classified into 1 of 3 groups: 1) GnRH-induced ovulation-SF (< 12 mm; Ind-SF; n = 10); 2) GnRH-induced ovulation-LF (> 13 mm; Ind-LF; n = 43); or 3) spontaneous ovulation-LF (> 13 mm; Spon-LF; n = 27). The increase in P4 concentrations was greater ( $P = 0.06$ ) in pregnant (d 2 to 12) compared with nonpregnant cows. Also, the increase in P4 from d 2 to 12 was greater ( $P = 0.01$ ) in the Ind-LF compared with the Ind-SF groups, but there was no difference ( $P = 0.94$ ) among groups in P4 from d 14 to 60 in pregnant cows. Follicle size at AI influenced the increase in P4 in cows that failed to conceive ( $P = 0.007$ ), but not among cows that became pregnant ( $P = 0.32$ ) to AI. In summary, P4 secretion after GnRH-induced ovulation of SF was decreased from d 2 to 12 compared with that of LF, but was similar among pregnant cows from d 14 to 60 postAI (d 0).

**Key words:** beef cow, estradiol, follicle size, ovulation

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## INTRODUCTION

In postpartum beef cows, ovulatory follicle size varied (< 11 to > 16 mm) at spontaneous estrus or at GnRH-induced ovulation (CO-Synch protocol; Geary et al., 2001). Cows that had an ovulatory follicle > 12 mm at GnRH-induced ovulation had greater pregnancy rates compared with cows induced to ovulate follicles ≤ 12

mm (Lamb et al., 2001). Recent studies indicate GnRH-induced ovulation of follicles ≤ 11 mm decreased pregnancy rates and increased the incidence of late embryonic/fetal mortality (Perry et al., 2005). However, spontaneous ovulation of follicles ≤ 11 mm had no effect on pregnancy rate or embryonic/fetal survival. Consequently, GnRH-induced ovulation of physiologically immature follicles can reduce pregnancy rate and late embryonic/fetal survivability (Perry et al., 2005).

It is currently unknown whether the cause of late embryonic/fetal mortality after ovulation of a physiologically immature follicle in postpartum beef cows is due to oocyte incompetence, inadequate uterine environ-

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ment, or both. It is also unknown whether GnRH-induced ovulation of different size dominant follicles affects subsequent progesterone secretion in nonlactating beef cows. The hypothesis was that GnRH-induced ovulation of a small dominant follicle would result in formation of luteal tissue that produced decreased concentrations of progesterone through the time of placental attachment compared with large dominant follicles in which ovulation was induced with GnRH or follicles that ovulated spontaneously.

Therefore, the specific objectives were as follows: 1) to determine the effect of ovulatory follicle size at GnRH-induced or spontaneous ovulation on serum concentrations of progesterone in nonlactating beef cows (Exp. 1), and 2) to determine the effect of ovulatory follicle size at GnRH-induced or spontaneous ovulation on serum concentrations of progesterone (d 2 to 60) and estradiol (d -2 to 0) in lactating beef cows through the time of placental attachment and the previously reported period of embryonic loss (d 27 to 41; Perry et al., 2005; Exp. 2).

## MATERIALS AND METHODS

The experimental procedures were approved by the University of Missouri—Columbia Animal Care and Use Committee.

### Experiment 1

**Animals and Treatments.** Nonlactating, nonpregnant (2 to 12 yr old) crossbred beef cows ( $n = 64$ ) at the USDA-ARS, Fort Keogh Livestock and Range Research Laboratory in Miles City, MT, were treated with the CO-Synch protocol. Cow body condition (mean  $\pm$  SEM =  $4.8 \pm 0.08$ ; 1 = emaciated; 9 = obese) was determined at the beginning of the experiment. The experiment was designed as a  $2 \times 2$  factorial, based on whether the cows were induced to ovulate with GnRH or allowed to spontaneously ovulate and on ovulatory follicle size (small,  $\leq 11$  mm, or large,  $\geq 12$  mm). Cows were allotted to the GnRH-induced or spontaneously ovulating groups; however, if the animals allotted to the GnRH-induced group showed estrus before the second GnRH injection they were transferred to one of the spontaneously ovulating groups.

The treatment groups were as follows: GnRH-induced ovulation of a small dominant follicle ( $\leq 11$  mm; **Ind-SF**;  $n = 9$ ); GnRH-induced ovulation of a large dominant follicle ( $\geq 12$  mm; **Ind-LF**;  $n = 16$ ); spontaneous ovulation of a small dominant follicle ( $\leq 11$  mm; **Spon-SF**;  $n = 8$ ); or spontaneous ovulation of a large dominant follicle ( $\geq 12$  mm; **Spon-LF**;  $n = 22$ ). Perry et al. (2005) illustrated that ovulation of optimally sized follicles was required for maximal pregnancy rates within a herd of cows, but that optimal size appears to differ between herds. Cows received GnRH (100  $\mu$ g as 2 mL of OvaCyst i.m.; Phoenix Scientific, St. Joseph, MO) on the first day of treatment (d -9), and PGF<sub>2 $\alpha$</sub>  (25 mg as

5 mL of ProstaMate i.m.; Phoenix Scientific) on d -2. Forty-eight hours after PGF<sub>2 $\alpha$</sub> , cows not detected in estrus received a second injection of GnRH (OvaCyst; 100  $\mu$ g i.m.; d 0). Cows that exhibited estrus [d 0, as determined by HeatWatch Estrous Detection System (DDx Inc., Denver, CO);  $\geq 3$  mounts  $\geq 2$  s in duration within a 4-h period] after the PGF<sub>2 $\alpha$</sub>  injection were allowed to spontaneously ovulate. Ovulation was assumed to have occurred if serum concentrations of progesterone were greater than 0.5 ng/mL by 7 d postGnRH/estrus (Schafer et al., 2007). Cows were removed (Ind-SF = 4, Ind-LF = 2, Spon-SF = 0, and Spon-LF = 3) from the study if they exhibited a short estrous cycle ( $\leq 14$  d;  $n = 7$ ), did not undergo complete luteal regression ( $n = 1$ ), or had circulating concentrations of progesterone that did not reach 1 ng/mL for more than 3 d during the luteal phase ( $n = 1$ ).

**Blood Sample Collection.** Blood samples were collected via venipuncture (coccygeal vein) into 10-mL Vacutainer tubes (Fisher Scientific, Pittsburgh, PA) daily from the time of PGF<sub>2 $\alpha$</sub>  injection of the CO-Synch protocol to the subsequent estrus (as determined by using the HeatWatch Estrous Detection System). Blood was allowed to clot at room temperature, stored at 4°C for 24 h, and centrifuged at  $2,000 \times g$  for 20 min. Serum was harvested and stored at -20°C until serum concentrations of progesterone were determined by RIA.

**Ultrasonography.** Ovaries of all cows were examined by transrectal ultrasonography to characterize follicular development at the time PGF<sub>2 $\alpha$</sub>  was administered and at the time of the second GnRH injection or approximately 3 h after the onset of estrus (as determined by using the HeatWatch Estrous Detection System) using an Aloka 500V ultrasound with a 7.5-MHz transrectal linear probe (Aloka, Wallingford, CT). Follicular size was determined by measuring follicular diameter at the widest point of the follicle and at a right angle to the first measurement using the internal calipers of the Aloka 500V ultrasound. The preceding 2 measurements were averaged to obtain follicular diameter, and average follicular diameter was rounded to the nearest millimeter.

**Radioimmunoassay.** Serum concentrations of progesterone were analyzed in all serum samples by RIA (Bellows et al., 1991; Diagnostic Products Corporation, Los Angeles, CA). Intra- and interassay CV for progesterone were 11.9 and 11.2%, respectively, and assay sensitivity was 0.08 ng/mL of serum.

**Statistical Analysis.** Serum concentrations of progesterone were analyzed by analysis of variance for repeated measures (PROC MIXED; Littell et al., 1998; SAS Inst. Inc., Cary, NC). The statistical model included treatment (Ind-SF, Ind-LF, Spon-SF, and Spon-LF), day, and their interactions. Analysis of the increase in progesterone (d 3 to 15) after GnRH-induced or spontaneous ovulation was analyzed by using PROC MIXED of SAS. After regression of progesterone on day, differences in the slopes were analyzed.

## Experiment 2

**Animals and Treatments.** Postpartum, suckled (2 to 16 yr old), crossbred beef cows ( $n = 99$ ) at the University of Missouri—Columbia Beef Farm (mean days postpartum = 76.4; range 36 to 99 d) were treated with the CO-Synch protocol (Geary et al., 2001). The experiment was designed as a  $2 \times 2$  factorial based on whether ovulation was induced with GnRH or allowed to occur spontaneously and on ovulatory follicle size (small;  $\leq 12$  mm or large;  $\geq 13$  mm). Only 2 cows spontaneously ovulated a small follicle; therefore, they were excluded from the analysis of the effect of treatment on serum concentrations of progesterone. Consequently, orthogonal contrasts were performed as follows: GnRH-induced ovulation of a large follicle vs. GnRH-induced ovulation of a small follicle, and GnRH-induced vs. spontaneous ovulation of a large follicle. However, the 2 cows that spontaneously ovulated a small follicle and the 5 cows that ovulated 12.5-mm follicles were included in the analysis of the effect of follicle size (10.0 to 12.5 mm; 13.0 to 14.5 mm; and  $\geq 15.0$  mm) at the time of AI on serum concentrations of progesterone from d 2 to 12 after GnRH or estrus.

The treatment groups were as follows: GnRH-induced ovulation of a small dominant follicle ( $\leq 12$  mm; Ind-SF;  $n = 10$ ); GnRH-induced ovulation of a large dominant follicle ( $\geq 13$  mm; Ind-LF;  $n = 43$ ); or spontaneous ovulation of a large dominant follicle ( $\geq 13$  mm; Spon-LF;  $n = 27$ ). Because the optimally sized follicle was 12.8 mm for Missouri cows (Exp. 1 of Perry et al., 2005) and 11.3 mm for Montana cows (Exp. 2 of Perry et al., 2005), we chose  $\leq 11$  and  $\geq 12$  for Montana cows (Exp. 1) and  $\leq 12$  and  $\geq 13$  for Missouri cows (Exp. 2) in the current study to differentiate between small and large ovulatory size. Cows received GnRH (100  $\mu$ g as 2 mL of Cystorelin i.m.; Merial, Athens, GA) at the initiation of treatment (d -9) and PGF<sub>2 $\alpha$</sub>  (25 mg as 5 mL of Lutalyse i.m.; Pfizer, New York, NY) on d -2. Forty-eight hours after PGF<sub>2 $\alpha$</sub> , cows received an injection of GnRH (Cystorelin; 100  $\mu$ g i.m.; d 0) and received AI, by an experienced technician, with semen from 1 of 2 bulls. Any cows that showed estrus (as determined by using the HeatWatch Estrous Detection System;  $\geq 3$  mounts  $\geq 2$  s in duration within a 4-h period) before PGF<sub>2 $\alpha$</sub>  or the second GnRH injection received AI approximately 12 h after the onset of estrus.

Cow body condition (1 = emaciated; 9 = obese) was determined at the time of the first blood sample (see below). Cows were considered anestrus if they had serum concentrations of progesterone less than 1 ng/mL in 2 blood samples collected 10 d before and at the time of the initial GnRH injection. Cows were considered to be estrous cycling if serum concentrations of progesterone were greater than 1 ng/mL in at least one of the blood samples collected before the first GnRH injection (Perry et al., 2005). Calves were maintained with the cows at all times and were allowed to suckle without restriction. Cows were removed ( $n = 11$ ) from the study

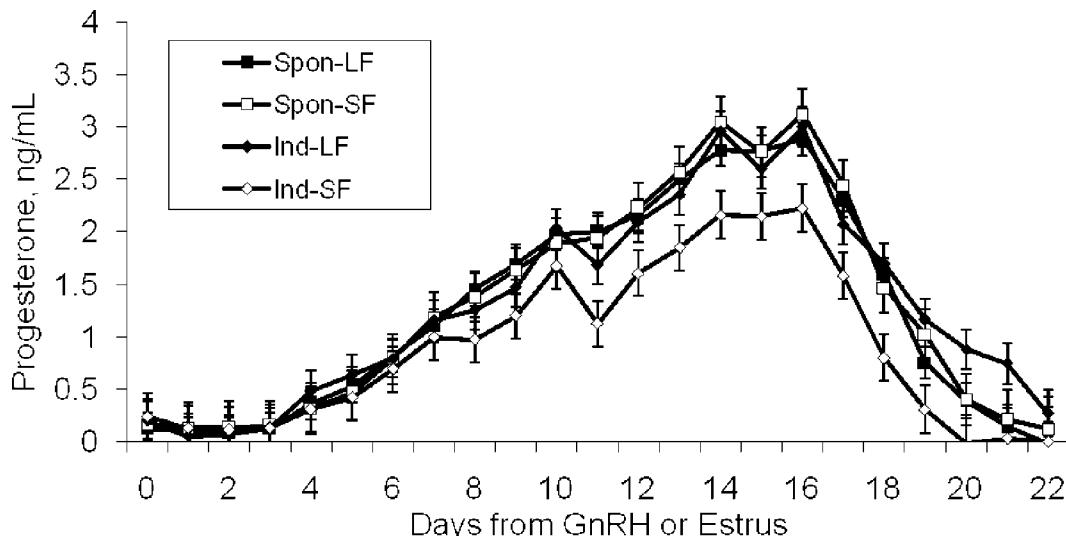
if they did not ovulate in response to the GnRH injection at the time of insemination ( $n = 5$ ), ovulated 2 follicles in response to the GnRH injection at the time of insemination ( $n = 5$ ), or were culled based on disposition ( $n = 1$ ).

**Blood Sample Collection.** Blood samples were collected via jugular venipuncture into 10-mL Vacutainer tubes (Fisher Scientific) 10 d before the initiation of treatment and on the first day of treatment to determine estrous cycling status. Additional serum samples were collected on d -2, -1, and 0 (d 0 = AI) and every other day after insemination to determine serum concentrations of estradiol (d -2, -1, and 0) and progesterone (d 2 to 60). Blood was allowed to clot at room temperature, stored at 4°C for 24 h, and centrifuged at 2,000  $\times g$  for 20 min. Serum was harvested and stored at -20°C until serum concentrations of estradiol and progesterone were determined by RIA.

**Ultrasonography.** Ovaries of all cows were examined by transrectal ultrasonography to characterize follicular development (d -2 and 0; d 0 = AI) and ovulation 3 d after AI using an Aloka 500V ultrasound with a 7.5-MHz transrectal linear probe. All follicles  $\geq 8$  mm diam. were recorded. Follicle size was determined as described in Exp. 1 by measuring follicular diameter at the widest point of the follicle and at a right angle to the first measurement using the internal calipers on the Aloka 500V ultrasound. These 2 measurements were averaged to obtain follicular diameter. Ovulation was defined as the disappearance of a large ( $\geq 9$  mm) follicle from an ovary after spontaneous estrus or GnRH administration (d 0). Beginning on d 28 after insemination, the uteri of all cows were examined every 8 d by transrectal ultrasonography to determine pregnancy status and embryo viability (heartbeat) using an Aloka 500V ultrasound with a 5-MHz transrectal linear probe until d 60 of gestation.

**Assays.** Serum concentrations of progesterone were analyzed by RIA (Kirby et al., 1997; Diagnostic Products Corporation). Intra- and interassay CV for progesterone assays were 2.7 and 8.7% respectively, with an assay sensitivity of 0.1 ng/mL. Serum estradiol concentrations were determined by RIA in serum samples collected on d -2, -1, and 0 (Kirby et al., 1997). Intra- and interassay CV for estradiol-17 $\beta$  assays were 10.6 and 12.5%, respectively, with an assay sensitivity of 0.25 pg/mL.

**Statistical Analysis.** Serum concentrations of progesterone and estradiol-17 $\beta$  were analyzed by analysis of variance for repeated measures (PROC MIXED; Littell et al., 1998) using SAS. The statistical model consisted of the variable tested (treatment and pregnancy status), day, and their interactions. Analysis of the increase in progesterone (d 2 to 12) after GnRH-induced or spontaneous ovulation was by PROC MIXED of SAS, and the statistical model included treatment (Ind-SF, Ind-LF, and Spon-LF), day, and their interactions. In addition, the effect of follicle size groups (10.0 to 12.5 mm; 13.0 to 14.5 mm; and  $\geq 15.0$  mm) at the time of AI on the rate of increase in serum concentrations of



**Figure 1.** Mean serum concentrations of progesterone (bars = SEM) from d 0 to 22 postGnRH or estrus (d 0) for nonlactating cows that experienced spontaneous ovulation of a large ( $\geq 12$  mm) dominant follicle (Spon-LF;  $n = 22$ ), spontaneous ovulation of a small (10 to 11 mm) dominant follicle (Spon-SF;  $n = 8$ ), GnRH-induced ovulation of a large dominant follicle (Ind-LF;  $n = 16$ ), or GnRH-induced ovulation of a small dominant follicle (Ind-SF;  $n = 9$ ) in Exp. 1. There was an effect of day ( $P = 0.01$ ) and a treatment  $\times$  day interaction ( $P = 0.05$ ) on mean serum concentrations of progesterone from d 3 to 15 postGnRH or estrus. There was also an effect of treatment ( $P < 0.01$ ), day ( $P < 0.01$ ), and a treatment  $\times$  day interaction ( $P < 0.01$ ) on the increase in progesterone from d 3 to 15.

progesterone (d 2 to 12) was also analyzed by PROC MIXED of SAS, and the model included follicle size group, day, and their interactions. After regression of progesterone on day, differences in the slopes were analyzed. Orthogonal contrasts were performed between the Ind-SF and Ind-LF groups and the Ind-LF and Spon-LF groups.

The effects of treatment, age, days postpartum, BCS, cyclicity, and AI sire on d 28 pregnancy rates were determined by categorical ANOVA using PROC GENMOD of SAS. When the  $F$ -statistic was significant ( $P < 0.05$ ), mean ( $\pm$  SEM) separation was performed using least significant differences (Snedecor and Cochran, 1989).

## RESULTS

### Experiment 1

There was no overall effect of treatment ( $P = 0.14$ ; Ind-SF, Ind-LF, Spon-SF, and Spon-LF) on mean serum concentrations of progesterone from d 3 to 15 after ovulation. However, there was an effect of day ( $P < 0.01$ ) and a treatment  $\times$  day interaction ( $P = 0.05$ ) on mean serum concentrations of progesterone (Figure 1). Cows in the Ind-SF group had lower ( $P < 0.05$ ) serum concentrations of progesterone on d 12 to 15 compared with the Ind-LF, Spon-LF, and Spon-SF groups. There was an effect of treatment ( $P < 0.01$ ), day ( $P < 0.01$ ), and treatment  $\times$  day interaction ( $P < 0.01$ ) on the increase in serum concentrations of progesterone from d 3 to 15. The increase in serum concentrations of progesterone

was reduced from d 3 to 15 in Ind-SF compared with the Ind-LF ( $P = 0.05$ ), Spon-SF ( $P = 0.07$ ), and Spon-LF ( $P = 0.03$ ).

### Experiment 2

There was no difference among treatments in cow age, days postpartum, BCS or estrous cyclicity status at the initiation of treatment (Table 1). Pregnancy rates in the Ind-SF, Ind-LF, and Spon-LF groups were 70, 72, and 70%, respectively ( $P = 0.93$ ) and only 1 cow lost an embryo (Ind-LF group) through d 60. In addition, there was no effect of age ( $P = 0.21$ ), days postpartum ( $P = 0.75$ ), treatment groups ( $P = 0.93$ ), or AI sire ( $P = 0.22$ ) on pregnancy rates at d 28 postinsemination. There was an effect of BCS ( $P = 0.02$ ) and cyclicity ( $P = 0.03$ ) on pregnancy rates 28 d postinsemination. Cows with a BCS of 4.5 to 5.0, 5.5 to 6.0, and  $\geq 6.5$  had pregnancy rates on d 28 of gestation of 44, 78, and 78%, respectively. Pregnancy rates on d 28 postinsemination in cows that were anestrous or cycling at the start of treatment were 45 and 75%, respectively.

There was an effect of treatment ( $P = 0.04$ ), day ( $P < 0.01$ ), and treatment  $\times$  day interaction ( $P < 0.01$ ) on serum concentrations of progesterone from d 2 to 12 postAI (Figure 2). The increase in serum concentrations of progesterone from d 2 to 12 was greater ( $P = 0.01$ ) in the Ind-LF group compared with the Ind-SF group. However, the increase in concentrations of progesterone from d 2 to 12 was similar ( $P = 0.89$ ) when the Ind-LF and Spon-LF groups were compared. Pregnancy status affected the increase ( $P = 0.06$ ) in serum concen-

**Table 1.** Number of cows, age, days postpartum, BCS, and estrous cycling status for cows before the initiation of treatment (mean  $\pm$  SEM; Exp. 2)

Treatment <sup>1</sup>	No.	Age, yr	Days postpartum <sup>2</sup>	BCS <sup>3</sup>	Cows with elevated progesterone <sup>4</sup>	
					Proportion	Percentage
Ind-LF	43	5.5 $\pm$ 0.3	78.7 $\pm$ 0.5	5.9 $\pm$ 0.1	36/43	84
Ind-SF	10	4.9 $\pm$ 0.6	73.9 $\pm$ 1.4	5.6 $\pm$ 0.2	8/10	80
Spon-LF	27	5.8 $\pm$ 0.3	75.1 $\pm$ 0.6	6.0 $\pm$ 0.2	25/27	93

<sup>1</sup>Treatment groups were determined at the time follicular diameter was measured at AI. Ind-LF = GnRH-induced ovulation of a large ( $\geq 13$  mm) dominant follicle at AI; Ind-SF = GnRH-induced ovulation of a small ( $\leq 12$  mm) dominant follicle at AI; Spon-LF = spontaneous ovulation of a large ( $\geq 13$  mm) dominant follicle after onset of estrus.

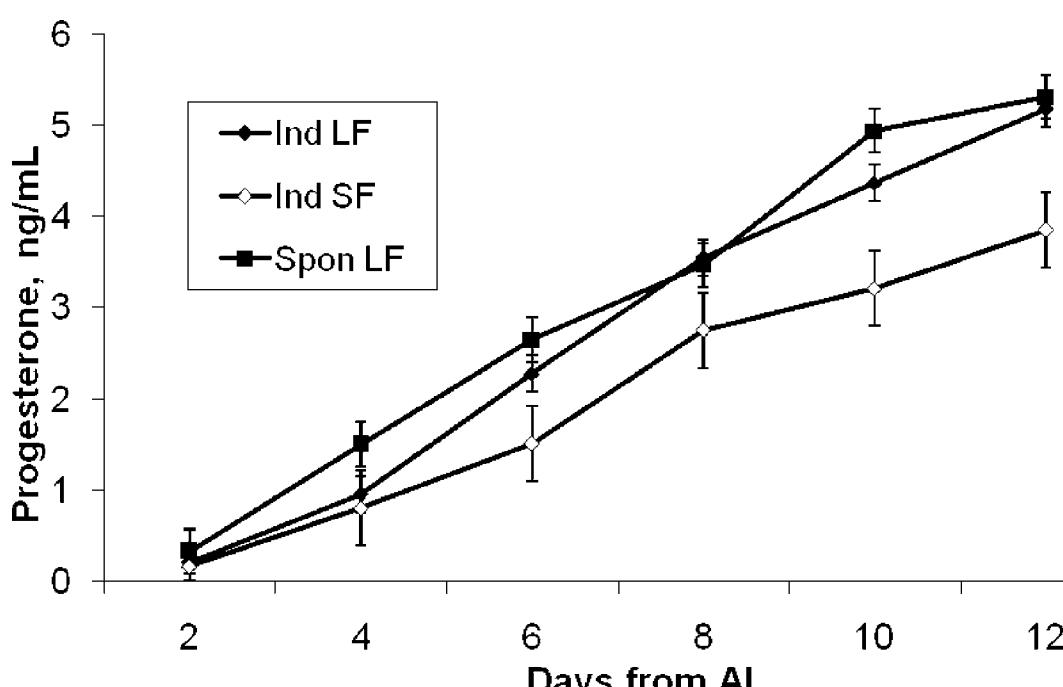
<sup>2</sup>Number of days postpartum at the initiation of the CO-Synch protocol.

<sup>3</sup>Body condition score (1 to 9 scale, where 1 = emaciated and 9 = obese) of cows at the time of the first blood sample before the initiation of treatment.

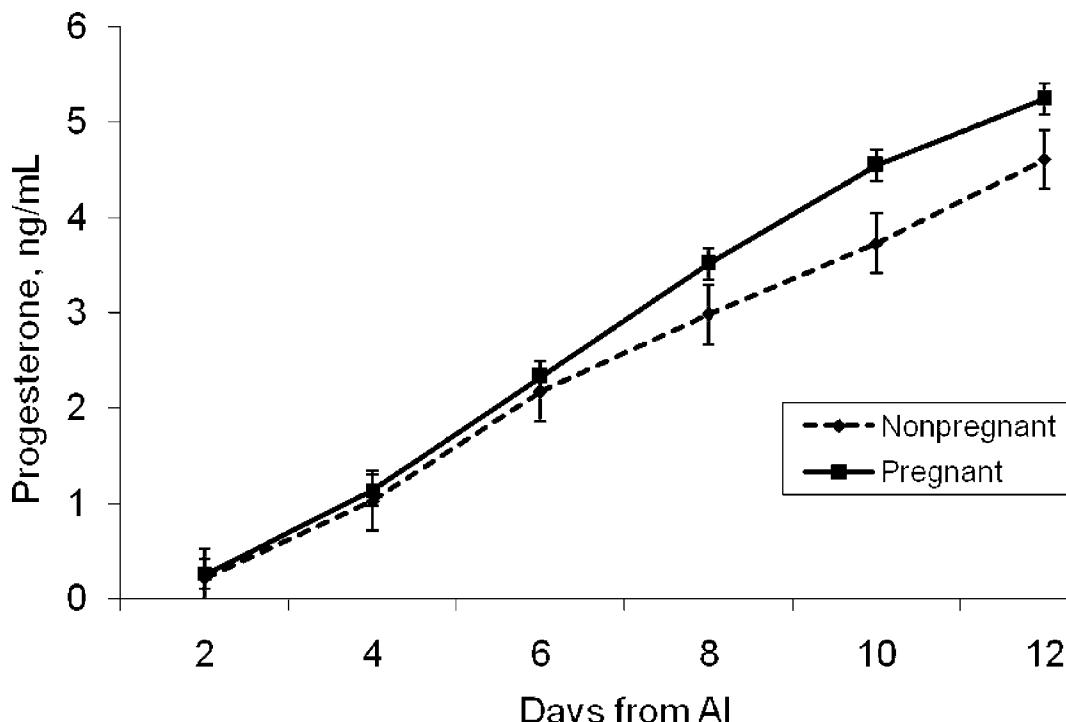
<sup>4</sup>Estrous cyclicity = the percentage of cows with elevated ( $\geq 1$  ng/mL) concentrations of progesterone in serum before treatment. Cows were considered to be cyclic if progesterone was elevated in either of 2 blood samples collected 10 d before and at the initiation of treatment.

trations of progesterone from d 2 to 12 (Figure 3). Therefore, treatment groups were analyzed according to AI pregnancy status. There was no difference in serum concentrations of progesterone from d 2 to 12 ( $P = 0.24$ ) or from d 2 to 60 ( $P = 0.24$ , Figure 4) among treatments in cows that conceived to AI (as determined by ultrasound 28 d after AI). Also, the increase in serum concentrations of progesterone from d 2 to 12 in pregnant cows was similar between the Ind-LF and Ind-SF group ( $P =$

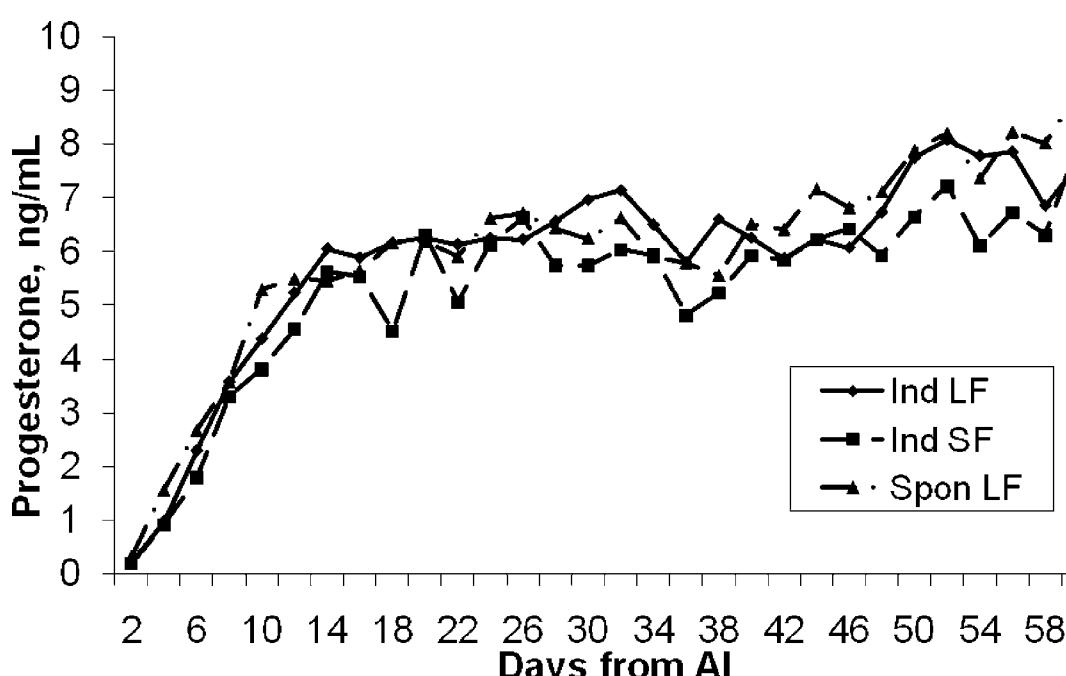
0.26) as well as between spontaneous ovulation and GnRH-induced ovulation ( $P = 0.65$ , Figure 4). However, in cows that did not conceive to AI, induced ovulation of a large dominant follicle (Ind-LF) resulted in an elevated increase ( $P < 0.01$ ) in progesterone when compared with Ind-SF (Figure 5). Alternatively, there was no difference ( $P = 0.35$ ) in the increase in serum concentrations of progesterone between the Ind-LF and Spon-LF groups in cows that did conceive to AI. Additionally,



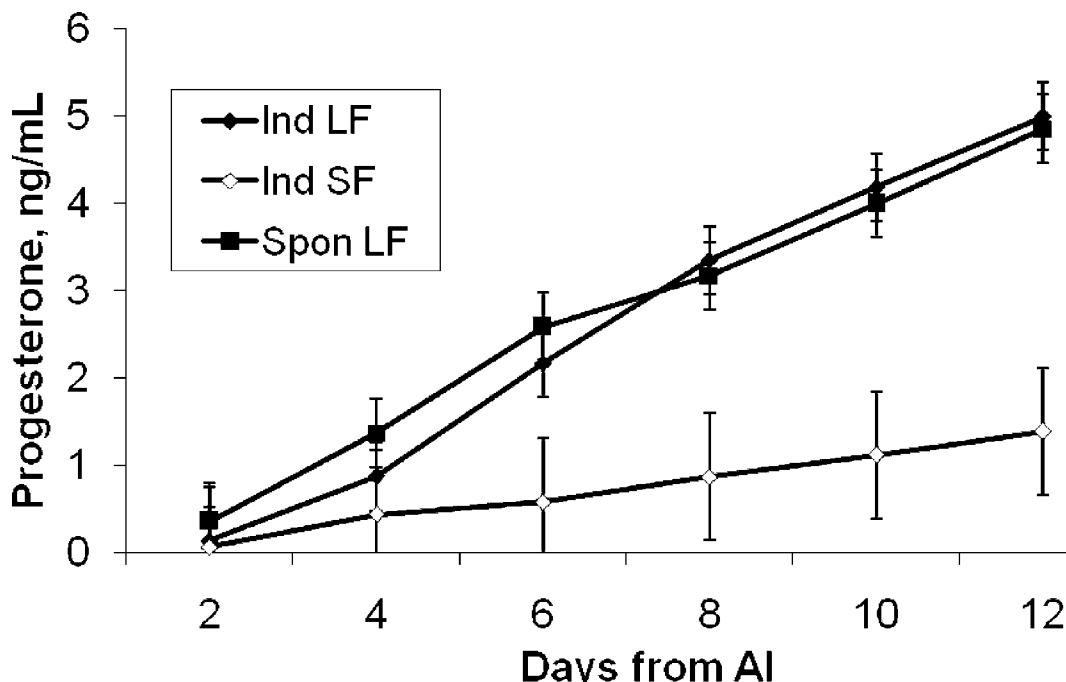
**Figure 2.** Mean serum concentrations of progesterone (bars = SEM) for lactating beef cows from d 2 to 12 postAI (d 0 = AI) after the GnRH-induced ovulation of a large follicle (Ind LF; n = 43), GnRH-induced ovulation of a small follicle (Ind SF; n = 10), or spontaneous ovulation of a large follicle (Spon-LF; n = 27) in Exp. 2. There was an effect of treatment on serum concentrations of progesterone from d 2 to 12 (treatment,  $P < 0.05$ ; day,  $P < 0.01$ ; treatment  $\times$  day,  $P < 0.01$ ) and a difference in the increase in concentration of progesterone from d 2 to 12 between the Ind-LF and Ind-SF groups ( $P = 0.01$ ; GnRH-induced vs. spontaneous ovulation of a large follicle,  $P = 0.89$ ).



**Figure 3.** Mean serum concentrations of progesterone (bars = SEM) for pregnant ( $n = 64$ ) and nonpregnant ( $n = 24$ ) lactating beef cows from d 2 to 12 postAI (d 0 = AI) in Exp. 2. There was an effect of pregnancy status on the increase in concentrations of progesterone from d 2 to 12. Pregnant cows had greater ( $P = 0.02$ ) circulating concentrations of progesterone than nonpregnant cows beginning on d 10 after AI.



**Figure 4.** Mean serum concentrations of progesterone (bars = SEM) in pregnant cows from d 2 to 60 postAI (d 0 = AI) after the GnRH-induced ovulation of a large follicle (Ind LF;  $n = 31$ ), GnRH-induced ovulation of a small follicle (Ind SF;  $n = 7$ ), or spontaneous ovulation of a large follicle (Spon-LF;  $n = 19$ ) in Exp. 2. There was no effect of treatment ( $P = 0.24$ ) or treatment  $\times$  day interaction ( $P = 0.52$ ) on mean serum concentrations of progesterone.



**Figure 5.** Mean serum concentrations of progesterone (bars = SEM) in nonpregnant cows from d 2 to 12 postAI (d 0 = AI) after the GnRH-induced ovulation of a large follicle (Ind LF; n = 12), GnRH-induced ovulation of a small follicle (Ind SF; n = 3), or spontaneous ovulation of a large follicle (Spon-LF; n = 8) in Exp. 2. There was an effect of treatment ( $P = 0.01$ ) and day ( $P < 0.01$ ) on mean serum concentrations of progesterone, and there also were differences in the increase in progesterone (Ind-LF vs. Ind-SF,  $P < 0.01$ ; GnRH-induced vs. spontaneous ovulation of a large follicle,  $P = 0.35$ ).

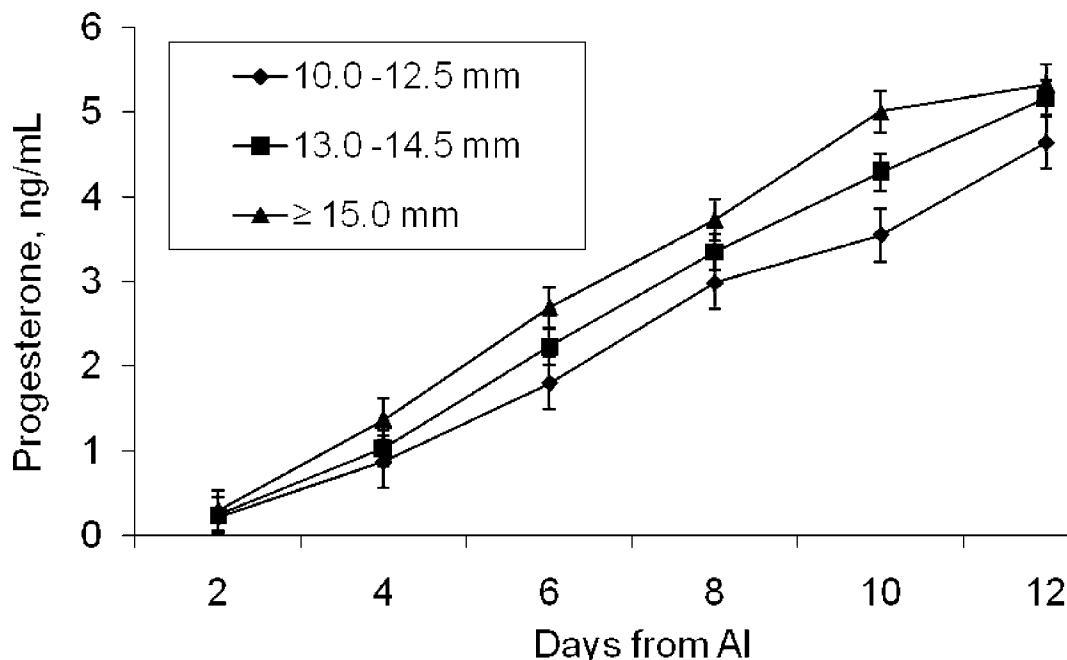
follicle size groups (10.0 to 12.5 mm; 13.0 to 14.5 mm; and  $\geq 15.0$  mm) at the time of AI affected serum concentrations of progesterone from d 2 to 12 (follicle size,  $P = 0.07$ ; day,  $P < 0.01$ ; follicle size  $\times$  day,  $P = 0.02$ ; Figure 6). Cows ovulating follicles 10.0 to 12.5 mm had reduced ( $P = 0.02$ ) serum concentrations of progesterone compared with cows ovulating follicles  $\geq 15$  mm.

Preovulatory circulating concentrations of estradiol may be a measure of the physiological maturity of a dominant follicle; therefore, serum concentrations of estradiol were measured on d -2, -1, and 0. There was no effect ( $P = 0.43$ ) of treatment on serum concentrations of estradiol on d -2, -1, and 0 (d 0 = AI); however, there was a treatment  $\times$  day interaction ( $P < 0.01$ , Figure 7). Also, there was no difference ( $P = 0.77$ ) in serum concentrations of estradiol between cows that became pregnant to AI and cows that did not conceive to AI, but again there was a pregnancy  $\times$  day interaction ( $P = 0.03$ ). Further analysis of pregnancy status on d 28 revealed an effect of treatment on serum concentrations of estradiol in nonpregnant ( $P < 0.01$ ; Figure 8) but not pregnant cows ( $P = 0.90$ ; Figure 9).

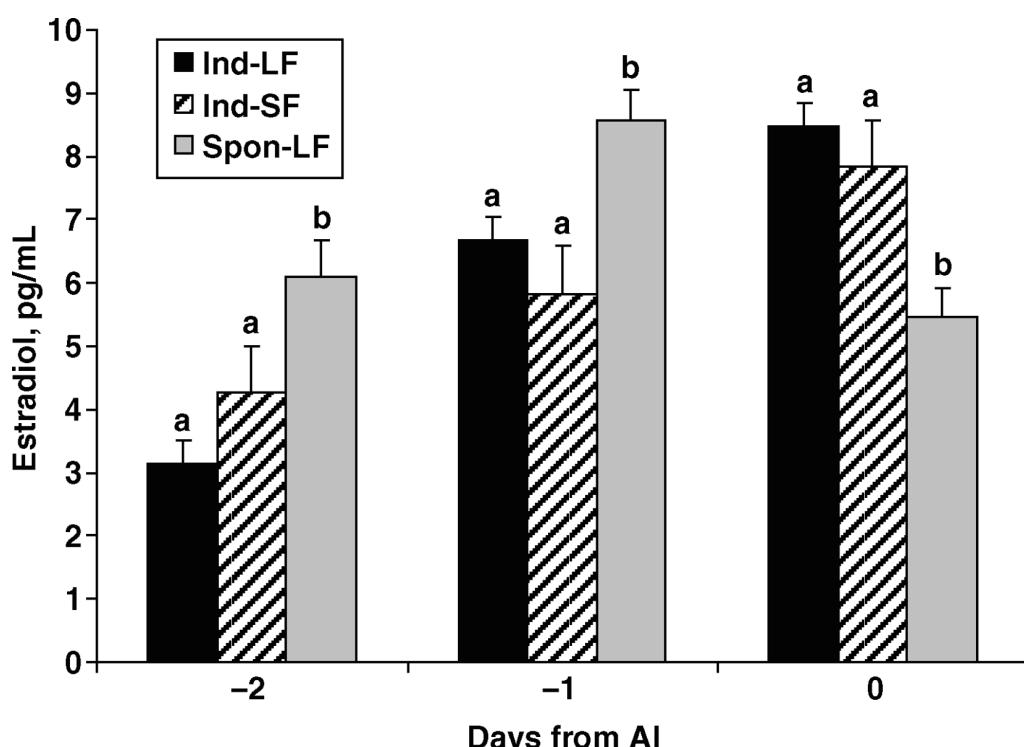
## DISCUSSION

The mechanism(s) by which dominant follicle size may decrease pregnancy rate and late embryonic/fetal survival in cows after GnRH treatment may be attrib-

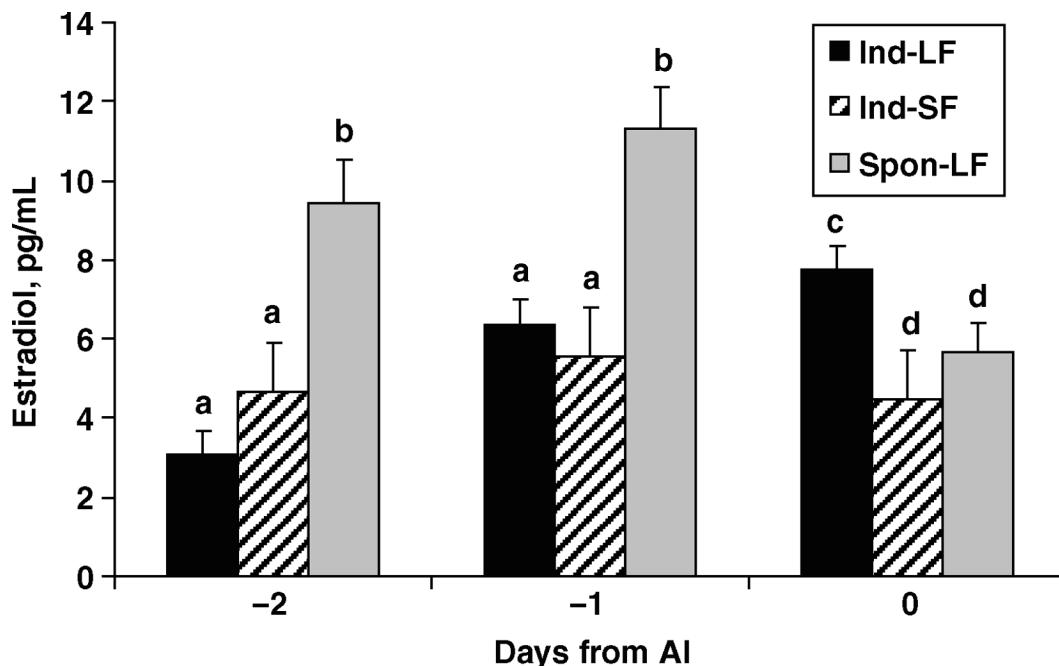
uted to ovulation of an incompetent oocyte, inadequate uterine environment, or both. As an initial step toward elucidating the preceding mechanism(s), the effect of GnRH-induced ovulation of small or large dominant follicles on pattern of secretion of progesterone was determined in nonlactating and lactating beef cows. In Exp. 1 and 2, cows that were induced to ovulate a small dominant follicle ( $\leq 11$  and  $\leq 12$  mm, respectively) with GnRH had decreased serum concentrations of progesterone from the time of ovulation to the plateau and a reduced rate of progesterone increase during the same time period compared with GnRH-induced ovulation of large follicles. Therefore, regardless of lactational status, GnRH induced ovulation of a physiologically immature follicle resulted in formation of CL in which production of progesterone was reduced. However, in Exp. 1, follicle size did not influence subsequent concentrations of progesterone when cows spontaneously ovulated. Therefore, it is not follicle size but the physiological maturity of the follicle that determined the subsequent function of the luteal tissue. Smaller follicles capable of spontaneous ovulation might have reduced numbers of granulosa/large luteal cells, but this does not exclude these follicular cells from secreting adequate amounts of progesterone after luteinization. Vasconcelos et al. (2001) also reported that induced ovulation of small follicles ( $11.5 \pm 0.2$  mm) resulted in development of smaller CL that secreted less progesterone



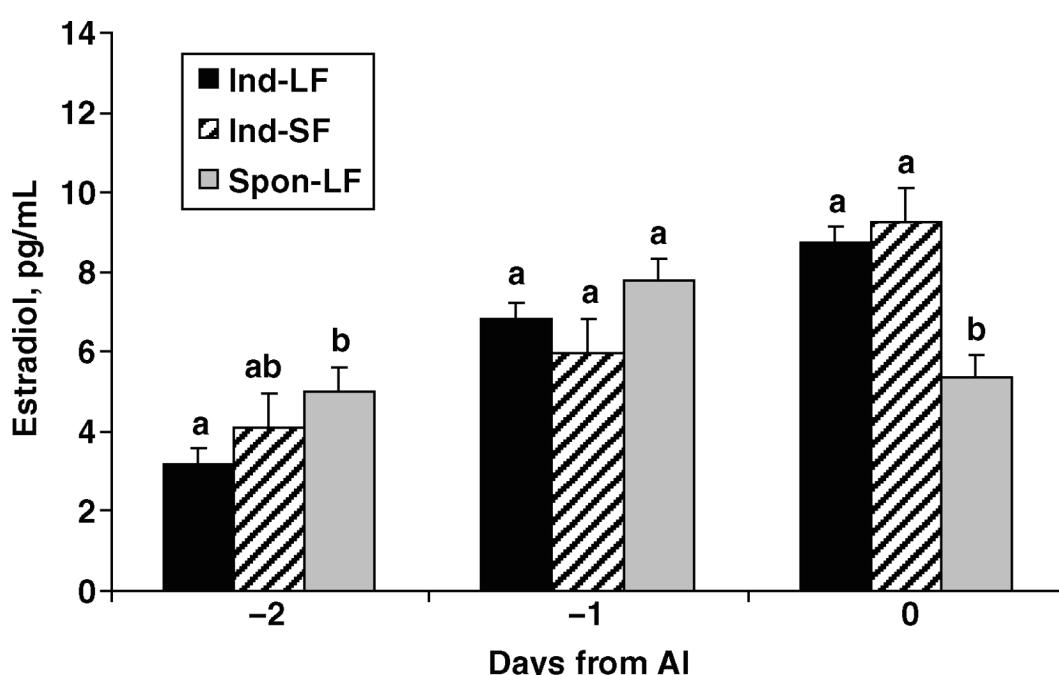
**Figure 6.** Effect of ovulatory follicle size [10.0 to 12.5 mm ( $n = 17$ ); 13.0 to 14.5 mm ( $n = 36$ ); and  $\geq 15.0$  mm ( $n = 28$ )] at AI (d 0) on mean serum concentrations of progesterone (bar = SEM) from d 2 to 12 postAI (follicle size,  $P = 0.07$ ; day,  $P < 0.01$ ; follicle size  $\times$  day,  $P = 0.02$ ) in Exp. 2. A difference in serum concentrations of progesterone among ovulatory follicle size groups was detected (10.0 to 12.5 mm vs.  $\geq 15.0$  mm,  $P = 0.02$ ).



**Figure 7.** Mean serum concentrations of estradiol-17 $\beta$  (bar = SEM) on d -2, -1, and on the day of AI (d 0) in lactating beef cows after the GnRH-induced ovulation of a large follicle (Ind LF;  $n = 43$ ), GnRH-induced ovulation of a small follicle (Ind SF;  $n = 10$ ), or spontaneous ovulation of a large follicle (Spon-LF;  $n = 27$ ) in Exp. 2. There was no effect of treatment, but there was an effect of day and a treatment  $\times$  day interaction on mean serum concentrations of estradiol-17 $\beta$  on d -2, -1, and 0 (treatment,  $P = 0.43$ ; day,  $P < 0.01$ ; treatment  $\times$  day,  $P < 0.01$ ). <sup>a,b</sup>Bars having different superscripts within a day are different ( $P < 0.05$ ).



**Figure 8.** Effect of treatment in nonpregnant cows on mean serum concentrations of estradiol-17 $\beta$  (bar = SEM) on d -2, -1, and on the day of AI (d 0; treatment,  $P < 0.01$ ; day,  $P < 0.01$ ; treatment  $\times$  day,  $P < 0.01$ ) after the GnRH-induced ovulation of a large follicle (Ind LF;  $n = 12$ ), GnRH-induced ovulation of a small follicle (Ind SF;  $n = 3$ ), or spontaneous ovulation of a large follicle (Spon-LF;  $n = 8$ ) in Exp. 2. Bars having different superscripts are different, <sup>a,b</sup>within d -2 and -1 ( $P < 0.01$ ), and <sup>c,d</sup>within d 0 ( $P < 0.05$ ).



**Figure 9.** Effect of treatment in pregnant cows on mean serum concentrations of estradiol-17 $\beta$  (bar = SEM) on d -2, -1, and on the day of AI (d 0; treatment,  $P = 0.99$ ; day,  $P < 0.01$ ; treatment  $\times$  day,  $P < 0.01$ ) after the GnRH-induced ovulation of a large follicle (Ind LF;  $n = 31$ ), GnRH-induced ovulation of a small follicle (Ind SF;  $n = 7$ ), or spontaneous ovulation of a large follicle (Spon-LF;  $n = 19$ ) in Exp. 2. <sup>a,b</sup>Bars having different superscripts within a day are different ( $P < 0.01$ ).

compared with induced ovulation of larger follicles ( $14.47 \pm 0.39$  mm) in dairy cows. Similarly, ovine follicles induced to ovulate 12 h after luteal regression had fewer granulosa cells and formed smaller CL that secreted less progesterone than follicles induced to ovulate 36 h after luteal regression (Murdoch and Van Kirk, 1998).

In the current study, pregnant cows had greater serum concentrations of progesterone beginning on d 10 after insemination compared with nonpregnant cows. Similar differences in circulating concentrations of progesterone as early as d 6 postinsemination have been reported in cows that became pregnant compared with cows that did not become pregnant (Mann et al., 1999; Perry et al., 2005). Cows that underwent an earlier rise in progesterone had embryos that were further developed and produced more antiluteolytic protein IFN- $\tau$  by d 16 than cows that had a delayed rise in progesterone (Mann and Lamming, 2001). These data indicate that exposure to an earlier rise in progesterone may contribute to increased embryonic/fetal survival.

There was no effect of treatment on mean serum concentrations of progesterone in pregnant cows from d 2 to 12 or from d 2 to 60 (Exp. 2). Therefore, reduced progesterone production after GnRH-induced ovulation of small dominant follicles was not observed in pregnant cows from d 0 to 60. If reduced serum concentrations of progesterone do not contribute to late embryonic/fetal mortality in postpartum cows in which small dominant follicles are induced to ovulate with GnRH, it is possible that inadequate induction of endometrial progesterone receptors by estradiol may have a role. The increased incidence of late embryonic/fetal mortality after GnRH-induced ovulation of small dominant follicles was associated with decreased serum concentrations of estradiol on the day of insemination (Perry et al., 2005), and estradiol has been associated with the induction of endometrial progesterone receptors (Zelinski et al., 1980). Inadequate uterine environment may provide an explanation for reduced pregnancy rates after GnRH-induced ovulation of small dominant follicles because transfer of embryos into recipient heifers that ovulated a 10-mm follicle maintained fewer pregnancies than heifers that ovulated 13-mm follicles (Mussard et al., 2003).

Estradiol may play a role in preparation of follicular cells for luteal formation and function. The ability of luteinized human granulosa cells to secrete progesterone was increased when cells were collected from follicles having increased follicular fluid concentrations of estradiol compared with granulosa cells collected from follicles with lower concentrations of estradiol (McNatty et al., 1979), and secretion of progesterone was delayed in ewes given an aromatase inhibitor before induced ovulation (Benoit et al., 1992). Therefore, cows that exhibited standing estrus and had greater serum estradiol concentrations during the 2 d before insemination may have attained concentrations of estradiol necessary to adequately prepare the follicular cells for luteinization, regardless of follicular size, or

induced an adequate number of uterine progesterone receptors, or both, as discussed earlier. Interestingly, estradiol concentrations 1 d before AI differed between cows that did or did not become pregnant. In nonpregnant cows on the day preceding insemination, serum concentrations of estradiol in the Ind-SF and Ind-LF groups were decreased compared with the Spon-LF group, whereas in the pregnant cows serum concentrations of estradiol were similar. The reduction in serum concentrations of estradiol on the day of insemination among cows that exhibited estrus and spontaneously ovulated is likely due to a decrease in aromatase activity and subsequent estradiol secretion after the endogenous gonadotropin surge. The decreased serum concentrations of estradiol in the nonpregnant cows preceding insemination may have affected oocyte viability, preparation of follicular cells for luteinization, induction of endometrial progesterone receptors, or a combination of these.

In summary, GnRH-induced ovulation of small follicles resulted in decreased serum concentrations of progesterone and a reduced rate of progesterone increase after ovulation. However, there was no effect of treatment on serum concentrations of progesterone from d 2 to 60 of gestation in cows that were diagnosed pregnant by ultrasonography on d 28 postinsemination. In contrast, serum concentrations of progesterone from d 2 to 12 postinsemination among nonpregnant cows were decreased after GnRH-induced ovulation of small follicles. Therefore, a possible mechanism by which GnRH-induced ovulation of small follicles reduces pregnancy rates may be through reduced progesterone concentrations during early pregnancy.

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