Influence of exogenous gonadotropin-releasing hormone on ovarian function in beef cows after short- and long-term nutritionally induced anovulation1,2

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ABSTRACT: The effect of pulsatile infusion of gonadotropin-releasing hormone (GnRH) on follicular function was evaluated in nutritionally induced anovulatory beef cows. After 4 (short; n = 12) or 18 wk (long; n = 12) of anovulation, cows were randomly assigned within anovulatory group to either 2 H9262 g of GnRH treatment or saline (control; i.v.) every hour for 5 d. Ovarian structures were monitored by daily ultrasonography. Growth rate of the largest follicle (P < 0.01) and maximal size of the largest follicle during treatment were greater (P < 0.01) for GnRH vs control cows. At exsanguination after 5 d of GnRH treatment, the size of the second-largest follicle was greater (P < 0.05) in short (i.e., 4 wk) anovulatory cows than in long (i.e., 18 wk) anovulatory cows and the largest follicle tended (P < 0.10) to be larger in long vs short anovulatory cows. Short anovulatory GnRH-treated cows had more small follicles than short anovulatory control cows or long anovulatory GnRH-treated or control cows (anovulation × GnRH; P < 0.10). Follicular fluid (FFL) concentrations of estradiol (P < 0.01) and androstenedione (P < 0.05) were greater in GnRH vs control cows. Concentrations of insulin-like growth factor-I were greater (P < 0.10) in large vs small follicles in cows that were anovulatory for 4 wk, but not in cows that were anovulatory for 18 wk. The amount of insulin-like growth factor-binding protein (IGFBP)-3 in FFL was greater (P < 0.05) in 4- vs 18-wk anovulatory cows. Amounts of IGFBP-2, -4, and -5 were greater (P < 0.001) in FFL of small (<5 mm) vs large (≥5 mm) follicles regardless of treatment. We conclude that pulsatile treatment with GnRH for 5 d stimulates similar growth of the largest follicles in short- and long-term anovulatory beef cows, and that the duration of anovulation is not a major factor that limits follicular growth when anovulatory cows are treated with GnRH. The primary intrafollicular factors associated with increased follicular size were increased concentrations of estradiol, progesterone, and insulin-like growth factor-I, and decreased concentrations of IGFBP-2, -4, and -5. Increased duration of anovulation was associated with decreased concentrations of IGF-I and IGFBP-3 in FFL.

Key Words: Anovulation, Beef Cows, Follicles, Gonadotropin-Releasing Hormone, Insulin-Like Growth Factor, Nutrition


Introduction

A principal cause of reduced reproductive performance in beef cattle is an extended anestrous period

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follicular dominance (Murphy et al., 1991; Bossis et al., 1999), and changes in IGFBP may be involved in the development of dominant follicles (de la Sota et al., 1996; Stewart et al., 1996; Mihm et al., 2000). Production of progesterone and androstenedione by bovine theca cells and estradiol by bovine granulosa cells in vitro are stimulated by IGF-I and inhibited by IGFBP-3 (Spicer et al., 1997; Spicer and Chamberlain, 1999).

A longer duration of anestrus in beef cows may decrease the ability of ovarian follicles to synthesize hormones and increase in size when exposed to gonadotropins. The objectives of this study were to determine the effect of nutritionally induced anovulation duration (4 or 18 wk) and pulsatile treatment with GnRH on follicular growth and concentrations of steroids, IGF-I, and IGFBP in follicular fluid.

**Material and Methods**

*Animals and Treatments.* Twenty-four nonlactating, Hereford × Angus cows (2 to 8 yr old; BW = 453.0 ± 10.2 kg; BCS = 5.0 ± 0.2, [1 = emaciated and 9 = obese; Wagner et al., 1988]) exhibiting normal estrous cycles were maintained in a drylot and fed a restricted diet to lose 1% of their initial BW/wk. Cows consumed 2.72 kg of prairie hay and 35 g of mineral mix/d. When ambient temperature was below 0 °C, an additional 1.4 kg of hay was provided each day. Body weight and BCS were determined every 14 d. Blood samples (10 mL) were collected every 7 d via venipuncture into tubes containing EDTA (0.1 mL of a 15% solution). Blood samples were immediately placed on ice, centrifuged (3,000 × g for 20 min) within 3 h of collection, and plasma was decanted and stored at −20 °C until progesterone was quantified. Cows were determined to be anovulatory when concentrations of progesterone in plasma were less than 1 ng/mL for three consecutive weeks.

At 4 (short; 3.7 ± 0.1 wk) or 18 wk (long; 18.1 ± 1.2 wk) after the onset of anovulation, cows were confined to individual stalls (21 ± 4°C) and fed a diet of prairie hay (5.5 kg) and mineral mix (35 g) every day at 0900. When present at the start of ultrasonography were <10 mm in diameter and grew more than 2 mm during the 5-d study. Cows were classified as initiating a new wave of follicular growth if either the largest follicle present at the start of ultrasonography was ≥10 mm in diameter and grew <2 mm during the 5-d study, or if no follicles larger than 5 mm were detected during the 5-d study.

After 5 d of treatment, cows were exsanguinated within 2 h after the cessation of GnRH treatment. Animals were killed with a captive-bolt device as approved by the Institutional Animal Care and Use Committee. Uteri and ovaries were removed, placed on ice, and transported to the laboratory. Follicles on the right and left ovaries were counted and diameters were measured with calipers; outlines of follicles were enhanced by using a bright light source behind the ovary. Follicular fluid was collected with 1-mL tuberculin syringes (Becton Dickinson & Co., Franklin Lakes, NJ). Follicular fluid was pooled within the ovary for small follicles (<5 mm) and collected from individual large follicles (≥5 mm). Follicular fluid was not centrifuged. Samples were stored at −20 °C until estradiol, progesterone, androstenedione, IGF-I, and IGFBP were quantified. Three cows that ovulated were deleted from analyses.

Concentrations of each hormone in plasma or follicular fluid were determined in a single assay. A solid-phase RIA (Coat-A-Count progesterone kit, Diagnostic Products Corp., Los Angeles, CA) was used to determine concentrations of progesterone in plasma (Vizcarra et al., 1997). The intraassay coefficient of variation was 3%. Estradiol concentrations in plasma were analyzed by RIA (estradiol MAIA; Polymedics, New York, NY) with modifications (Vizcarra et al., 1997); the intraassay coefficient of variation was 10%. Progesterone and estradiol in follicular fluid were quantified by RIA...
(Spicer and Enright, 1991), with intraassay coefficients of variation of 8% and 12%, respectively. Concentrations of IGF-I in follicular fluid were determined by RIA after an acid-ethanol extraction at 4°C for 16 h (Echternkamp et al., 1990), with an intraassay coefficient of variation of 10%. Concentrations of androstenedione in follicular fluid were determined by solid-phase RIA (ICN Biomedicals, Costa Mesa, CA), as described by Stewart et al. (1996), with an intraassay coefficient of variation of 11%.

A subset of 37 of 74 follicular fluid samples, one or two from each cow, were selected for quantification of IGFBP in follicular fluid via one-dimensional SDS-PAGE (Echternkamp et al., 1994; Spicer et al., 2001) based on: 1) if present, an individually collected large follicle with the greatest estradiol concentration from each cow, and/or 2) one or two small follicle pools (either the right or left ovary pool) randomly selected from each cow. Only 11 of 21 cows evaluated had both categories of follicular fluid samples analyzed. One cow had none of its follicular fluid samples analyzed for IGFBP and the other nine cows had either 1) only fluid from a pool (or pools) of small follicles analyzed for IGFBP (n = 6), or 2) fluid from a large follicle analyzed for IGFBP, other than the one with the greatest estradiol concentration (n = 3). In addition, this subset of follicles was selected in order to be able to run all samples at the same time (four gels) in the same electrophoresis chamber and hence minimize variation among gels. Buffer (21 μL) and 4 μL of sample were heat-denatured and then separated on a 12% PAGE (15 lanes per gel) via electrophoresis. Follicular fluid samples from 2 to 4 cows in each of the four groups (treatment/duration) were run on each gel. In addition to follicular fluid samples, colored molecular mass markers (Sigma Chemical Co.) and 4 μL of pooled bovine follicular fluid were used to identify band size and specific IGFBP. Proteins in each gel were electrophoretically transferred to nitrocellulose after separation and ligand-blotted for 17 h with bovine 125I-IGF-II. Gels were washed and exposed to X-ray film at −70°C for 24 h. The intensity of bands on autoradiographs was determined using scanning densitometry (Bio-Rad Molecular Imager and Imaging Densitometer, Bio-Rad Laboratories; Hercules, CA), and IGFBP activity was expressed as arbitrary densitometric units (ADU) per 4 μL of follicular fluid. Variation among gels was monitored via running the same bovine follicular fluid pool on each gel. The IGFBP-2, -3, and -5 bands were scanned and the resultant ADU for each IGFBP was used to calculate an intergel CV, which averaged 17.9 ± 5.2% for four gels.

**Statistical Analyses.** Follicular growth, maximal follicular size, and the number of cows that had one or more plasma samples with concentrations of estradiol greater than 1 pg/mL and progesterone greater than 1 ng/mL were analyzed by ANOVA using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC) with duration of anovulation (D), treatment (T), and D × T in the model. The number of follicles was analyzed by split-plot ANOVA with D, T, and D × T in the main plot, and ovarian side and the interactions with the main effects in the split-plot. Hormone concentrations in follicular fluid were analyzed by split-plot ANOVA with D, T, and D × T in the main plot, and follicular size (S) and the interactions with the main effects in the split-plot. The IGFBP in follicular fluid were analyzed as a 2 × 2 × 2 factorial ANOVA with D, T, S, and their interactions as main effects, and gel number was included as a covariable. If there were significant main effects or interactions, a Duncan test (Steel et al., 1997) was used to compare means. Because of heterogeneous variances (Steel et al., 1997), concentrations of estradiol, progesterone, androstenedione, and IGFBP-4 and -5 were transformed to natural log (x + 1) prior to analyses. Simple correlations (Steel et al., 1997) were calculated among IGF-I and IGFBP-2, -4, and -5. Percentage of cows initiating a new follicular wave was analyzed by χ² (Steel et al., 1997).

**Results**

**Ovarian Responses**

Consistent with previous results (Bishop and Wettmann, 1993; Vizcarra et al., 1997), cows became anovulatory when weight loss resulted in a BCS of 3.45 ± 0.03 and a BW of 350.8 ± 8.5 kg.

Rate of growth of the largest follicles, measured using ultrasonography, was not affected (P > 0.10) by duration of anovulation or the interaction between duration of anovulation and GnRH treatment. Rate of growth of the largest follicles during treatment with GnRH was greater (1.1 ± 0.1 mm/d; P < 0.002) compared to control cows (0.7 ± 0.2 mm/d). The percentage of cows initiating a new wave of follicular growth was 75 and 17% (P < 0.01) for GnRH- and saline-treated cows, respectively, during the 5-d treatment. The percentage of short- and long-term anovulatory cows initiating a new wave of follicular growth during the 5-d treatment was identical (50%). Three GnRH-treated cows (two short anovulatory cows and one long anovulatory cow) ovulated the last day of the treatment (d 5) before slaughter. Data from these cows were included in follicular growth analysis, but their data were not included in follicular fluid hormone analyses.

Maximal size of the largest follicle during treatment (detected via ultrasonography) was greater (P < 0.005) for GnRH (11.3 ± 0.9 mm) vs control cows (7.4 ± 0.5 mm) and was not affected by the duration of anovulation. Duration of anovulation influenced the size of the largest (P < 0.10) and second-largest follicle (P < 0.05) at exsanguinations, and GnRH-treatment had no effect. The largest follicle was 23% larger (P < 0.10) in cows that were long anovulatory than in cows that were short anovulatory. However, the second largest follicle was 30% larger (P < 0.05) in cows that were short anovulatory compared with cows that were long anovulatory.
Nutritionally induced anovulation

Figure 1. Influence of duration of anovulation on the size of the largest and second-largest ovarian follicles in nutritionally induced anovulatory cows at exsanguination. Short-term = 4 wk (n = 10); long-term = 18 wk (n = 11). a,bMeans differ (P < 0.10). c,dMeans differ (P < 0.05).

The number of large follicles was greater (P < 0.10) in the right ovary (1.2 ± 0.3 follicles) than in the left ovary (0.5 ± 0.2 follicles) and was not influenced by treatment or duration of anestrus.

The number of small (<5 mm) follicles in the ovaries at d 5 of GnRH treatment (exsanguination) was influenced by the duration of anovulation × treatment (P < 0.10). Gonadotropin-releasing hormone-treated cows that were short anovulatory had more small follicles (31.1 ± 4.0 follicles) than short anovulatory control cows (23.3 ± 2.9 follicles) or long GnRH or control cows (17.3 ± 5.0 and 24.3 ± 2.0 follicles, respectively). However, long control cows had more small follicles than long GnRH-treated cows.

Hormones in Plasma and Follicular Fluid

Concentrations of estradiol in plasma were not influenced by treatment. However, treatment with GnRH increased (P < 0.01) the percentage of cows with greater than 1 pg/mL of estradiol in plasma on one or more days during treatment. Eighty-three percent of cows treated with GnRH had greater than 1 pg/mL of estradiol on at least 1 d of treatment, whereas only 25% of the control cows had greater than 1 pg/mL of estradiol at any sampling time. The percentage of cows with concentrations of progesterone in plasma less than 1 ng/mL during the 5 d of treatment was not influenced by duration of anovulation or treatment; 83% of the treated cows and 92% of control cows had less than 1 ng/mL progesterone in all samples.

GnRH treatment (P < 0.01), size (P < 0.01), and their interaction (P < 0.08) affected concentrations of estradiol in follicular fluid (Figure 2). Large follicles of GnRH-treated cows had greater (P < 0.005) estradiol concentrations than small follicles (Figure 2) of GnRH-treated cows, and greater (P < 0.005) concentrations than large and small follicles of control cows. Of the largest follicles collected on d 5, only 25% of the control cows vs 56% of GnRH-treated cows (P < 0.05) had estrogen-active (i.e., estradiol > progesterone concentrations, Ireland and Roche, 1982; 1983) follicles. Duration of anovulation and its interaction with treatment or size had no effect (P > 0.10) on follicular fluid estradiol concentrations.

Duration of anovulation, size of follicles and their interaction did not influence (P > 0.10) concentrations of androstenedione in follicular fluid. Concentrations of androstenedione were greater (P < 0.05) in follicular fluid of GnRH-treated cows (79.8 ± 20.8 ng/mL) vs control cows (24.9 ± 18.8 ng/mL; Table 1). Duration of anovulation, GnRH treatment, and their interaction did not influence (P > 0.10) concentration of progesterone in follicular fluid. However, concentrations of progesterone in follicular fluid were greater (P < 0.001) in large follicles (166.7 ± 28.2 ng/mL) than in small follicles (48.8 ± 23.0 ng/mL).

There was a significant effect of duration of anovulation × follicular size on IGF-I in follicular fluid (P < 0.05; Table 1). Cows that were short anovulatory had 32% (P < 0.001) greater IGF-I concentrations in large follicles compared with small follicles, but concentrations of IGF-I were similar in large and small follicles of long anovulatory cows. Treatment with GnRH or its interaction with duration of anovulation or size did not influence (P > 0.10) concentration of IGF-I in follicular fluid.
Duration of nutritionally induced anovulation did not influence (P > 0.10) the concentrations of any of the IGFBP in follicular fluid. Follicular size did not influence the amount of IGFBP in follicular fluid. Follicular size did not influence the amount of IGFBP-3 (40- to 43-kDa IGFBP) activity; however, it was greater (39%; P < 0.05) in short anovulatory cows vs long anovulatory cows (Table 1). Follicular size influenced (P < 0.001) the amount of IGFBP-2 (34-kDa IGFBP), -4 (20- to 22-kDa IGFBP), and -5 (27- to 31-kDa IGFBP), but duration of anovulation did not influence (P > 0.10) the amounts of these IGFBP. Small follicles contained 2.5-fold more IGFBP-2, sevenfold more IGFBP-4, and 7.5-fold more IGFBP-3 compared with large follicles (Figure 3).

Table 1. Influence of duration of anovulation, treatment with GnRH (2 μg hourly), and follicular size on concentration of androstenedione, progesterone, IGF-I, and IGFBP-3 in follicular fluid

<table>
<thead>
<tr>
<th>Constituent in follicular fluid</th>
<th>Control</th>
<th>GnRH</th>
<th>Control</th>
<th>GnRH</th>
<th>MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androstenedione, ng/mL&lt;sup&gt;c&lt;/sup&gt;</td>
<td>≥5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;5</td>
<td>≥5</td>
<td>&lt;5</td>
<td>42.6</td>
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<tr>
<td>Progesterone, ng/mL&lt;sup&gt;d&lt;/sup&gt;</td>
<td>125.4</td>
<td>47.5</td>
<td>260.0</td>
<td>50.8</td>
<td>138.0</td>
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<td>IGF-I, ng/mL&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>8.70</td>
<td>12.85</td>
<td>10.32</td>
<td>9.26</td>
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<tr>
<td>IGFBP-3, ADU/4 μL&lt;sup&gt;f&lt;/sup&gt;</td>
<td>24.7</td>
<td>21.2</td>
<td>22.3</td>
<td>31.3</td>
<td>16.2</td>
</tr>
</tbody>
</table>

<sup>a</sup>Short-term = 4 wk; long-term = 18 wk.
<sup>b</sup>Size of follicle in millimeters.
<sup>c</sup>Treatment with GnRH (P < 0.06).
<sup>d</sup>Follicle size (P < 0.001).
<sup>e</sup>Duration of anovulation × follicle size (P < 0.05).
<sup>f</sup>ADU = Arbitrary densitometric units. Duration of anovulation (P < 0.05) and size (P < 0.07).
<sup>g</sup>Number of samples in parentheses.

Insulin-Like Growth Factor-Binding Protein in Follicular Fluid

Ligand blotting identified four forms of IGFBP in follicular fluid. Gonadotropin-releasing hormone treatment did not influence (P > 0.10) the concentrations of any of the IGFBP in follicular fluid. Follicular size did not influence the amount of IGFBP in follicular fluid. Follicular size did not influence the amount of IGFBP-3 (40- to 43-kDa IGFBP) activity; however, it was greater (39%; P < 0.05) in short anovulatory cows vs long anovulatory cows (Table 1). Follicular size influenced (P < 0.001) the amount of IGFBP-2 (34-kDa IGFBP), -4 (20- to 22-kDa IGFBP), and -5 (27- to 31-kDa IGFBP), but duration of anovulation did not influence (P > 0.10) the amounts of these IGFBP. Small follicles contained 2.5-fold more IGFBP-2, sevenfold more IGFBP-4, and 7.5-fold more IGFBP-3 compared with large follicles (Figure 3).

Discussion

Duration of nutritionally induced anovulation did not affect the rate of growth of the largest follicles, and the growth of large follicles in control cows was similar to that previously reported for nutritionally induced anestrous heifers (Bossis et al., 1999, 2000). Follicular waves occur in nutritionally induced anovulatory cows, but the largest follicles fail to mature and ovulate (Rhodes et al., 1995; Bossis et al., 2000). Beef heifers fed to lose body weight for 10 wk continued to ovulate, but had decreased persistence and decreased maximal diameter of the dominant follicle compared with heifers fed to gain body weight (Murphy et al., 1991). Thus, the specific component of follicular growth that is altered by nutrition may depend on the duration and severity of nutritional restriction.

In the present study, the number of small (<5 mm) follicles was increased by 5-d GnRH treatment in short-term, but not long-term, anovulatory cows, which is consistent with the notion that follicular turnover is greater in short- vs long-term anovulatory cows. In contrast, treatment of short-term nutritionally induced anovulatory cows with 2 μg of GnRH every hour for 12 d did not affect the number of follicles (Hamilton et al., 1999). This discrepancy is likely due to the fact that ovulation had occurred 3 d before collection of ovaries in cows of Hamilton et al. (1999), and thus a new wave of follicle growth had just been initiated at the time measurements were made. Similarly, numbers of follicles were not affected by GnRH (500 ng of GnRH every 2 h for 96 h) treatment of postpartum anestrous cows (Spicer et al., 1986a).

The number of large (but not small) follicles was greater in the right ovary than in the left ovary regard-
Figure 3. Influence of follicle size and GnRH treatment (2 μg/h), for 5 d on IGFBP-2, -4, and -5 levels in follicular fluid of small (<5 mm; saline, n = 14; GnRH, n = 11) and large (≥5 mm; saline, n = 6; GnRH, n = 6) follicles of nutritionally induced anovulatory cows. a,b Within a panel, means without a common letter differ (P < 0.05).
large (≥6.0 mm) (but not small, 1 to 5.9 mm) follicles (Spicer et al., 1992). When nutritionally induced anovulatory cows were realimentated with hay and a protein supplement, 88% of cows had short luteal phases before the first normal cycle; since only 28% of the cows exhibited estrus before the short luteal phase, this indicates that follicles may produce inadequate estradiol for normal cycles (Wettemann and Bossis, 2000). Collectively, the present and previous results indicate nutritional intake and GnRH treatment may affect estradiol concentrations in follicular fluid.

In contrast to the present study, Spicer et al. (1986b) reported that GnRH injections (500 ng every 2 h) for 2 or 4 d had no effect on follicular fluid androstenedione concentrations in postpartum anestrous beef cows. Similarly, Hamilton et al. (1999) found that treatment of nutritionally induced anovulatory cows with 2 µg of GnRH hourly or every 4 h for 12 d did not alter concentrations of androstenedione in follicles. Androgens are precursors for follicular estrogen production (Hillier, 1981) and theca cells are the major source of follicular androstenedione (McNatty et al., 1984b). Thus, it is likely that increased androgen synthesis by theca cells contributed to increased estradiol concentrations in follicles of the present study. Concentrations of androstenedione in follicular fluid of dominant and preovulatory follicles of cyclic cattle average between 200 and 500 ng/mL (Stewart et al., 1996; Spicer et al., 2001) and are much greater than concentrations measured in nutritionally induced anestrous cattle of the present and previous (Hamilton et al., 1999) studies. Whether the limited production of estradiol by follicles was due to smaller follicles or limited androstenedione production will require further elucidation.

Concentrations of progesterone in follicular fluid normally increase from d 2 to 10 of the bovine estrous cycle as follicular size increases during the first follicular wave (Bodensteiner et al., 1996; Stewart et al., 1996). Also, the ability of bovine follicles to produce progesterone appears to increase near the time of ovulation (Ireland and Roche 1982, 1983; Fortune and Hansel 1985), and both granulosa and theca cells are able to produce large amounts of progesterone (McNatty et al., 1984a; Spicer et al., 1993; Spicer and Francisco, 1998). Large follicles had greater concentrations of progesterone compared with small follicles, regardless of the duration of nutritional anovulation and GnRH treatment in the present study. Similarly, treatment with GnRH (2 µg) hourly or every 4 h for 12 d had no effect on follicular fluid progesterone concentrations in nutritionally induced anovulatory cows (Hamilton et al., 1999). On the other hand, treatment of postpartum beef cows with 500 ng of GnRH for 4 d decreased concentrations progesterone in follicular fluid of large follicles, but did not affect progesterone concentrations in small or medium follicles (Spicer et al., 1986b). This latter observation indicates that GnRH induced a wave of new follicle development (Spicer et al., 1986b). Whether GnRH treatment of the present study initiated a wave of new follicle growth or merely prolonged development of the existing dominant follicle is unclear, but based on the ultrasound data, more of the GnRH-treated cows than control cows initiated a new wave of follicular growth during the 5-d treatment.

Insulin-like growth factor-I could be a mediator of nutritional effects on reproduction because plasma concentrations of IGF-I are reduced in heifers (Spicer et al., 1992; Bossis et al., 1999, 2000) and cows (Spicer et al., 1991; Richards et al., 1995) subjected to underfeeding. Ryan et al. (1994) found that serum and follicular fluid concentrations of IGF-I in beef cows increased with increasing body condition. Increases in follicular fluid IGF-I concentrations occur as follicular diameter increases from small (1 to 4 mm) to medium or large (≥8 mm) follicles during the normal estrous cycle in cattle (Spicer and Enright, 1991). Concentrations of IGF-I were significantly increased in large vs small follicles of only short-term anovulatory cows in the present study. Similar concentrations of IGF-I in large and small follicles of long-term anovulatory cows could influence follicular function although not detected by the characteristics measured in this experiment. A gradual increase in IGF-I concentration in plasma is associated with a gradual increase in the size of dominant follicles during refeeding of nutritionally induced anovulatory heifers (Bossis et al., 2000). Also, cows selected for double ovulations and twin births have twofold greater concentrations of IGF-I in serum and follicular fluid than single-ovulating cows (Echternkamp et al., 1990). Collectively, these studies indicate that systemic IGF-I concentrations may influence growth of antral follicles of cattle.

Cattle have decreased concentrations of IGFBP-2, -4, and -5, with no change in IGFBP-3 in follicular fluid as follicles develop and produce estrogens (Echternkamp et al., 1994; Stewart et al., 1996; Spicer et al., 2001). Similarly, activity of IGFBP-2, -4, and -5 in follicular fluid was significantly greater in small vs large follicles regardless of the duration of anovulation and GnRH treatment in the present study. Although correlations between concentrations of estradiol and IGFBP-2 (r = −0.46), -4 (r = −0.38), and -5 (r = −0.31) were significant, comparing data in Figures 2 and 3 indicates that differences in estradiol concentrations cannot totally explain differences in concentrations of IGFBP between groups of large and small follicles. Similarly, follicular fluid levels of IGFBP-3 were not affected by GnRH in the present and previous studies (Hamilton et al., 1999), but were greater in short- vs long-term anovulatory cows regardless of follicular size. Because IGFBP-3 is not proteolyzed by bovine follicles (Spicer et al., 2001) or produced by granulosa cells from small follicles (Chamberlain and Spicer, 2001), it is likely that changes in follicular fluid levels of IGFBP-3 in the present study reflect changes in systemic levels of IGFBP-3, as previously suggested (Echternkamp et al., 1994).

In summary, concentrations of androstenedione, estradiol, progesterone, and IGFBP-2, -4, and -5 were
similar in follicles of cows 4 and 18 wk after nutritionally induced anovulation, although the largest follicle had lower concentrations of IGF-I and IGFBP-3 and a greater diameter in long- vs short-term anovulatory cows. Growth rate of the largest follicle and concentrations of androstenedione and estradiol in follicular fluid were increased similarly in long- and short-term anovulatory cows by hourly treatment with GnRH for 5-d. Large follicles had greater concentrations of progesterone and IGF-I and less IGFBP-2, -4, and -5 than small follicles.

**Implications**

Restriction of nutritional intake induces anovulation in beef cattle and affects reproductive efficiency by causing cessation of estrous cycles. Pulsatile treatment with gonadotropin-releasing hormone for 5 d stimulates follicular growth and ovarian steroidogenesis in nutritionally induced anovulatory cows regardless of how long (4 or 18 wk) the animals have been anovulatory. This indicates that duration of anovulation is not a major factor that limits the reestablishment of follicular growth and ovulation when anovulatory cows are treated with gonadotropin-releasing hormone or repleted. The primary intrafollicular factors associated with increased follicle size was decreased insulin-like growth factor-binding protein-2, -4 and -5 levels. Further work is needed to understand the endocrine, paracrine, and/or autocrine factors that regulate these insulin-like growth factor-binding proteins.

**Literature Cited**


indicus heifers before and after nutritional anoestrus. J. Reprod. Fertil. 104:41–49.


