Nutritionally Induced Anovulation in Beef Heifers: Ovarian and Endocrine Function Preceding Cessation of Ovulation

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ABSTRACT: Angus × Hereford heifers were used to determine endocrine and ovarian function preceding nutritionally induced anovulation. Six heifers were fed to maintain body condition score (M), and 12 heifers were fed a restricted diet (R) until they became anovulatory. Starting on d 13 of an estrous cycle, heifers were given PGF2α every 16 d thereafter to synchronize and maintain 16 d estrous cycles. Ovarian structures of M and R heifers were monitored by ultrasonography daily from d 8 to ovulation (d 1 of the subsequent cycle) until R heifers became anovulatory. Concentrations of LH and FSH were quantified in serum samples collected every 10 min for 8 h on d 2 and 15 (48 h after PGF2α), and estradiol and IGF-I were quantified in daily plasma samples from d 8 to 16 during the last ovulatory cycle (Cycle −2) and the subsequent anovulatory cycle (Cycle −1). During the last two cycles before anovulation, M heifers had 50% larger (P < .0001) ovulatory follicles than R heifers and 61% greater (P < .0001) growth rate of the ovulatory follicles. There was a treatment × cycle × day effect (P < .001) for concentrations of estradiol. The preovulatory increase in estradiol occurred in the R and M heifers during Cycle −2 but only in M heifers during Cycle −1. A treatment × cycle × day effect (P < .05) influenced LH concentrations. During Cycle −2, LH concentrations were similar for M and R heifers, but during Cycle −1, M heifers had greater LH concentrations than did R heifers. Concentrations of FSH were greater (P < .05) in R than M heifers after induced luteolysis when R heifers failed to ovulate. There was a treatment × cycle interaction (P < .05) for IGF-I concentrations, and M heifers had 4.7- and 8.6-fold greater IGF-I concentrations than did R heifers during Cycle −2 and −1, respectively. We conclude that growth rate and diameter of the ovulatory follicle, and concentrations of LH, estradiol, and IGF-I are reduced before the onset of nutritionally induced anovulation in beef heifers.

Key Words: Heifers, Nutrition, Ovaries, LH, Estradiol


Introduction

Nutrition is a major factor determining reproductive efficiency of beef cattle. Reduced nutrient intake delays the onset of puberty in heifers (Short and Bellows, 1971; Day et al., 1986; Yelich et al., 1995) and increases the postpartum interval to conception in beef cows (Wiltbank et al., 1964; Richards et al., 1986; Selk et al., 1988). Prolonged restriction of dietary energy in cattle results in loss of body weight and body condition, and cessation of estrous cycles (Richards et al., 1989a; Rhodes et al., 1996).

Restricted feed intake in beef cattle suppresses LH secretion (Day et al., 1986; Richards et al., 1989a; Yelich et al., 1995), reduces IGF-I (Richards et al., 1991) and glucose concentrations, and increases plasma GH and NEFA (Breier et al., 1986; Yelich et

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al., 1995). Restricted energy intake by heifers may have a direct effect on LH release (Beal et al., 1978). Reduced nutrient intake in cyclic (Murphy et al., 1991) and prepubertal (Bergfeld et al., 1994) beef heifers decreased persistence and maximum size of dominant follicles. A linear reduction in persistence and maximum size of dominant follicles occurred with decreasing body weight and condition score when feed was restricted for beef heifers (Rhodes et al., 1995).

Alterations in metabolic hormones, such as GH, insulin, and IGF-I, and blood metabolites, such as glucose and NEFA, are indicative of energy availability and may provide signals that mediate the effects of undernutrition on the hypothalamic-pituitary-ovarian axis. Knowledge about endocrine and metabolic changes associated with nutritionally induced anestrus will allow development of management and feeding systems to enhance reproductive efficiency of beef cattle. Our objectives were to evaluate follicular growth and concentrations of LH, FSH, and GH in serum and progesterone, estradiol, IGF-I, insulin, glucose, and NEFA in plasma during the last two cycles before the onset of nutritionally induced anovulation in beef heifers.

**Materials and Methods**

Angus × Hereford heifers with normal estrous cycles and moderate to good body condition scores (n = 18; BCS = 5.4 ± 2; 1 = emaciated, 9 = obese, Wagner et al., 1988; BW = 378 ± 15 kg) were used. In two replications, a total of six heifers were individually fed to maintain BCS (M group), and 12 heifers were individually fed a restricted diet (R group) to lose 1% of their BW per week (Table 1). Twice as many heifers were assigned to the R as to the M group to produce a sufficient number of anovulatory heifers to subsequently evaluate ovarian and endocrine function when two realimentation diets were fed. Body weight and BCS were recorded every 2 and 4 wk, respectively.

At the initiation of the study, estrous cycles of all heifers were synchronized with two injections of PGF$_{2\alpha}$ (Lutalyse®, 25 mg; Pharmacia & Upjohn, Kalamazoo, MI) with an 11-d interval between injections. Starting on d 13 of the synchronized estrous cycle, all heifers were given PGF$_{2\alpha}$ every 16 d for 11 to 17 estrous cycles to synchronize and maintain 16-d estrous cycles until R heifers became anovulatory. Transrectal ultrasonography was performed daily with an Aloka 500-V ultrasound instrument equipped with a 7.5-MHz transducer (Corometrics Medical Systems, Wallingford, CT) every second estrous cycle to monitor ovaries from 6 or 7 d after ovulation until the next ovulation (d 1 of the subsequent cycle). During ultrasonography, precise position and size of follicles and corpora lutea (CL) in both ovaries were sketched. Scans of the ovaries were also recorded on a video tape and viewed later to draw complete ovarian maps recording all follicles ≥ 4 mm and CL. Reference points on the ovaries included the poles, the hilus, and CL (Pierson et al., 1988). Diameters of follicles and CL were calculated as the mean of the longest and shortest diameters. The diameter of CL with fluid-filled cavities was estimated by subtracting the diameter of the cavity from the diameter of the entire CL (Savio et al., 1988). Day of emergence of the ovulatory follicle was defined retrospectively as the day before the first day that the ovulatory follicle could be individually identified. Growth rate of the ovulatory follicle was estimated as the increase in diameter from the day of emergence to the maximum diameter divided by number of days of growth. After R heifers had lost 12% of their initial body weight, ultrasonography was performed every cycle until heifers became anovulatory. During the estrous cycles when ultrasonography was performed, blood samples were collected every 10 min for 8 h on d 2 (1 d after ovulation) and d 15 (48 h after PGF$_{2\alpha}$). The day before sampling, a polyvinyl cannula (Bolab Inc., BB 317-V/11, i.d. 1.68 mm, o.d. 2.39 mm, Lake Havasu City, AZ) was inserted into a jugular vein of each heifer, and animals were confined to stalls. Blood samples (10 mL) were allowed to clot for 24 h at 4°C and centrifuged (2,800 × g, 30 min); serum was stored at −20°C until analyzed. Concentrations of LH, FSH, and GH were determined in serum collected every 10 min for 8 h on d 2 and 15 during the last ovulatory estrous cycle (Cycle −2) and the subsequent anovulatory estrous cycle (Cycle −1). A daily blood sample was collected before heifers consumed feed from d 8 until ovulation. Blood sampling was via tail venipuncture into 15 mL tubes containing

### Table 1. Composition of diets fed to heifers to maintain (maintenance) or lose (restricted) body condition

<table>
<thead>
<tr>
<th>Item</th>
<th>Maintenance</th>
<th>Restricted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredients, as fed, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rolled corn</td>
<td>37.5</td>
<td>—</td>
</tr>
<tr>
<td>Cottonseed hulls</td>
<td>21.7</td>
<td>—</td>
</tr>
<tr>
<td>Alalfa pellets</td>
<td>32.5</td>
<td>—</td>
</tr>
<tr>
<td>Prairie hay</td>
<td>—</td>
<td>96</td>
</tr>
<tr>
<td>Cane molasses</td>
<td>3.0</td>
<td>—</td>
</tr>
<tr>
<td>SBM</td>
<td>5.0</td>
<td>3</td>
</tr>
<tr>
<td>Limestone 38%</td>
<td>—</td>
<td>.6</td>
</tr>
<tr>
<td>Salt</td>
<td>.3</td>
<td>.3</td>
</tr>
<tr>
<td>Zinc oxide</td>
<td>—</td>
<td>.002</td>
</tr>
<tr>
<td>Vitamin A, 30,000</td>
<td>—</td>
<td>.04</td>
</tr>
<tr>
<td>Vitamin E, 50%</td>
<td>—</td>
<td>.04</td>
</tr>
<tr>
<td>Calculated values, as fed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>kg</td>
<td>4.5</td>
<td>4.0</td>
</tr>
<tr>
<td>DM %</td>
<td>88.9</td>
<td>89.8</td>
</tr>
<tr>
<td>Total NE m, Mcal</td>
<td>6.7</td>
<td>3.7</td>
</tr>
<tr>
<td>Total NE p, Mcal</td>
<td>3.6</td>
<td>1.7</td>
</tr>
<tr>
<td>CP %</td>
<td>12.2</td>
<td>6.7</td>
</tr>
</tbody>
</table>
prolactin. Tubes were placed on ice and centrifuged within 1 h (2,800 × g, 15 min), and plasma was stored at −20°C until analyzed. Concentrations of estradiol, glucose, insulin, IGF-I, and NEFA were quantified in daily plasma samples collected from d 8 until 16, and progesterone was quantified in daily samples from d 8 until 15 during estrous cycles. Estradiol concentrations in plasma were determined using the pulsar program (Merriam and Wachter, 1982). The G values used for LH were G1 = 99, G2 = 3.25, G3 = 2.75, G4 = 2.25, and G5 = 1.75; for FSH, G values were G1 = 99, G2 = 3.5, G3 = 3, G4 = 2.5, and G5 = 99; and for GH, G values were G1 = 99, G2 = 4.5, G3 = 4, G4 = 3.5, and G5 = 3. The G values were chosen to serve as criteria to determine whether variations in hormone concentrations in serial samples are pulses in hormone secretion or just random variations in concentrations.

Split-plot ANOVA were used to determine treatment effects on day of emergence, growth rate, and maximum diameter of the ovulatory follicle (or the anovulatory dominant follicle of the second wave in R heifers during estrous Cycle −1), and maximum CL diameter during the last two estrous cycles before anovulation. Treatment (M and R), replication (rep), and treatment × rep were the main plot, and cycle (−2 and −1), treatment × cycle, rep × cycle, and treatment × rep × cycle comprised the subplot. Mean square error (MSE) for heifer within treatment × rep was used as the error term for the main plot effects. The residual MSE was used as the error term for the subplot. Split-split-plot ANOVA were used to determine treatment effects on concentrations, pulse frequency, and amplitude of LH, FSH, and GH. Treatment, rep, and treatment × rep comprised the main plot; cycle, treatment × cycle, rep × cycle, and treatment × rep × cycle comprised the subplot; and day (d 2 and 15), treatment × day, cycle × day, rep × day, treatment × cycle × day, treatment × rep × day, rep × cycle × day, and treatment × cycle × rep × day comprised the sub-subplot. The MSE for heifer within treatment × rep was used as the error term for the main plot effects. Heifer within treatment × cycle × rep was the error term for the subplot. The residual MSE was used as the error term for the sub-subplot. Tukey-Kramer's procedure (unequal cell size) for pairwise comparisons was used to compare treatment means (SAS, 1990). Multivariate analyses of variance for repeated measures were used to determine treatment effects on progesterone, estradiol, IGF-I, insulin, glucose, and NEFA concentrations during the last two cycles before anovulation. Concentrations of hormones and metabolites from d 8 through 16 (d 8 to 15 for progesterone) during the last two cycles before anovulation were the repeated response variable (within-subject factors). The between-subject factors were treatment, rep, and treatment × rep in the main plot, and cycle, treatment × cycle, rep × cycle, and treatment × rep × cycle comprised the subplot. Because interactions of treatment with rep were either not significant or were due

EDTA (.1 mL of a 15% solution). Tubes were placed on ice and centrifuged within 1 h (2,800 × g, 15 min), and plasma was stored at −20°C until analyzed. Concentrations of estradiol, glucose, insulin, IGF-I, and NEFA were quantified in daily plasma samples collected from d 8 until 16, and progesterone was quantified in daily samples from d 8 until 15 during estrous cycles. Estradiol-17β concentrations in plasma were quantified with a RIA (Serono Estradiol MAIA assay kit, Biodata SpA, Montecelio, Italy) with modifications (Vizcarra et al., 1997). Intra- and interassay coefficients of variation (n = 6 assays) were 3 and 14%, respectively. Concentrations of LH in serum were quantified with a RIA (Bishop and Wachter, 1982). The G values used for LH were G1 = 99, G2 = 3.25, G3 = 2.75, G4 = 2.25, and G5 = 1.75; for FSH, G values were G1 = 99, G2 = 3.5, G3 = 3, G4 = 2.5, and G5 = 99; and for GH, G values were G1 = 99, G2 = 4.5, G3 = 4, G4 = 3.5, and G5 = 3. The G values were chosen to serve as criteria to determine whether variations in hormone concentrations in serial samples are pulses in hormone secretion or just random variations in concentrations.

Split-plot ANOVA were used to determine treatment effects on day of emergence, growth rate, and maximum diameter of the ovulatory follicle (or the anovulatory dominant follicle of the second wave in R heifers during estrous Cycle −1), and maximum CL diameter during the last two estrous cycles before anovulation. Treatment (M and R), replication (rep), and treatment × rep were the main plot, and cycle (−2 and −1), treatment × cycle, rep × cycle, and treatment × rep × cycle comprised the subplot. Mean square error (MSE) for heifer within treatment × rep was used as the error term for the main plot effects. The residual MSE was used as the error term for the subplot. Split-split-plot ANOVA were used to determine treatment effects on concentrations, pulse frequency, and amplitude of LH, FSH, and GH. Treatment, rep, and treatment × rep comprised the main plot; cycle, treatment × cycle, rep × cycle, and treatment × rep × cycle comprised the subplot; and day (d 2 and 15), treatment × day, cycle × day, rep × day, treatment × cycle × day, treatment × rep × day, rep × cycle × day, and treatment × cycle × rep × day comprised the sub-subplot. The MSE for heifer within treatment × rep was used as the error term for the main plot effects. Heifer within treatment × cycle × rep was the error term for the subplot. The residual MSE was used as the error term for the sub-subplot. Tukey-Kramer's procedure (unequal cell size) for pairwise comparisons was used to compare treatment means (SAS, 1990). Multivariate analyses of variance for repeated measures were used to determine treatment effects on progesterone, estradiol, IGF-I, insulin, glucose, and NEFA concentrations during the last two cycles before anovulation. Concentrations of hormones and metabolites from d 8 through 16 (d 8 to 15 for progesterone) during the last two cycles before anovulation were the repeated response variable (within-subject factors). The between-subject factors were treatment, rep, and treatment × rep in the main plot, and cycle, treatment × cycle, rep × cycle, and treatment × rep × cycle comprised the subplot. Because interactions of treatment with rep were either not significant or were due...
Nutritional treatment did not influence the interval to ovulation. During Cycle 2, 83% of M heifers ovulated 5 d after treatment. During Cycle −2, 83% of R and 83% of M heifers ovulated 4 d after PGF2α, and 17% of R and 17% of M heifers ovulated 5 d after PGF2α. During Cycle −1, all M heifers ovulated 4 d after PGF2α treatment, and none of the R heifers ovulated. Day of emergence of ovulatory follicles during Cycle −2 and Cycle −1 (anovulatory dominant follicle of the second wave in R heifers during Cycle −1) was not influenced by treatment (P > .1). Ovulatory follicles emerged on d 10.8 ± .4 of the estrous cycle for heifers on both treatments and were identified at a diameter of ≥ 4 mm (Table 3). Maintenance heifers had larger (P < .0001) ovulatory follicles (15.7 ± .9 mm) compared with R heifers (10.4 ± .9 mm) during the last two cycles before anovulation. Growth rate of the ovulatory follicle was greater (P < .001) for M (1.4 ± .1 mm/d) than R heifers (.9 ± .1 mm/d) in Cycles −2 and −1. Growth rate and diameter of the ovulatory follicle (or dominant follicle of the second wave in the anovulatory cycle) were not different between Cycle −2 and −1 in R heifers. In the M and R heifers, CL were maximal in diameter on d 13 of the cycle, but there was a treatment × cycle interaction (P < .05) for maximum CL diameter. Maintenance heifers had greater maximum CL diameter (19.7 ± .6 and 20.4 ± 1.0 mm for Cycle −2 and −1, respectively) than did R heifers (15.5 ± .4 and 14.1 ± .7 mm for Cycle −2 and −1, respectively) and CL diameter was less in Cycle −1 than in Cycle −2 in R heifers.

There was a treatment × day of cycle interaction (P < .05) for progesterone concentrations during the last two cycles before anovulation (Figure 1), but progesterone concentrations were similar between Cycle −2 and −1 in the R and M heifers. Concentrations of progesterone for the two treatments were best described by quintic regression equations (Figure 1). Analysis of heterogeneity of regression indicated that concentrations of progesterone for M heifers were greater than those in R heifers (P < .01).

There was a treatment × cycle × day interaction (P < .001) for concentrations of estradiol during the last two cycles before anovulation. During Cycle −2,
Table 4. Least squares means for concentration, pulse frequency, and pulse amplitude of LH and FSH during the last two estrous cycles before the onset of nutritionally induced anovulation.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Treatment</th>
<th>Maintenance</th>
<th>Restricted</th>
<th>MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cycle –2</td>
<td>Cycle –1</td>
<td>Cycle –2</td>
<td>Cycle –1</td>
</tr>
<tr>
<td></td>
<td>d 2  d 15</td>
<td>d 2  d 15</td>
<td>d 2  d 15</td>
<td>MSE</td>
</tr>
<tr>
<td>LH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration, ng/mL</td>
<td>5.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pulse frequency, pulses/8 h</td>
<td>3.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pulse amplitude, ng/mL</td>
<td>2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FSH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration, ng/mL</td>
<td>.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pulse frequency, pulses/8 h</td>
<td>1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pulse amplitude, ng/mL</td>
<td>.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means within a row without a common superscript differ (P < .05).

Table 5. Least squares means for concentration, pulse frequency, and pulse amplitude of GH during the last two cycles before the onset of nutritionally induced anovulation.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Treatment</th>
<th>Maintenance</th>
<th>Restricted</th>
<th>MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration of GH, ng/mL</td>
<td>12.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.8</td>
<td></td>
</tr>
<tr>
<td>Pulse frequency of GH, pulses/8 h</td>
<td>5.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Pulse amplitude of GH, ng/mL</td>
<td>17.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.4</td>
<td></td>
</tr>
</tbody>
</table>

Means within a row without a common superscript differ (P < .05).

Figure 1. Least squares means (MSE = 2.58) and least squares regression lines for progesterone concentrations during the last two cycles before the onset of nutritionally induced anovulation (PGF<sub>2α</sub> was given on d 13). Treatment x day interaction (P < .05).
Table 6. Least squares means for concentrations of glucose, insulin, IGF-I, and NEFA during the last two cycles before the onset of nutritionally induced anovulation

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Treatment</th>
<th>Cycle −2</th>
<th>Cycle −1</th>
<th>Cycle −2</th>
<th>Cycle −1</th>
<th>MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dL</td>
<td>Maintenance</td>
<td>70.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.8</td>
</tr>
<tr>
<td>Insulin, ng/mL</td>
<td>Restricted</td>
<td>1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
</tr>
<tr>
<td>IGF-I, ng/mL</td>
<td>Maintenance</td>
<td>91.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>31.7</td>
</tr>
<tr>
<td>NEFA, μEq/L&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Restricted</td>
<td>197&lt;sup&gt;a&lt;/sup&gt;</td>
<td>213&lt;sup&gt;a&lt;/sup&gt;</td>
<td>645&lt;sup&gt;b&lt;/sup&gt;</td>
<td>536&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4,060</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup>Means within a row without a common superscript differ (P < .05).
<sup>d</sup>μEq of palmitate.

greater (P < .0001) IGF-I concentrations than R heifers in Cycles −2 and −1 (Table 6). Concentrations of IGF-I in restricted heifers during Cycle −2 were greater than in Cycle −1.

Treatment influenced concentrations of glucose and insulin in plasma (P < .01). Cycle and day did not influence insulin and glucose concentrations. Concentrations of glucose and insulin were less in R heifers than in M heifers during Cycles −2 and −1 (Table 6).

There was a treatment × cycle interaction (P < .05) for NEFA concentrations. Restricted heifers had greater (P < .001) concentrations of NEFA than M heifers in Cycles −2 and −1, and concentrations of NEFA in R heifers during Cycle −2 were greater than in Cycle −1 (Table 6).

Discussion

A restriction in feed intake by R heifers resulted in loss of BW and BCS and failure of ovulation at an average of 32 wk after initiation of feed restriction. In agreement with our results, Richards et al. (1989a) found that beef cows became anestrous 26 wk after initiation of feed restriction, when cows had lost 24 and 36% of their initial BW and BCS, respectively. Similarly, Rhodes et al. (1996) found that beef heifers became anovulatory 23 wk after initiation of feed restriction, and this was accompanied by a 19% loss of initial BW.

Feed restriction reduced growth rate and maximum diameter of the ovulatory follicle during the last two cycles before anovulation. Reduced nutrient intake of cyclic (Murphy et al., 1991) and prepubertal (Bergfeld et al., 1994) beef heifers also resulted in decreased maximum diameter of the dominant follicle. A linear reduction in the maximum diameter of the dominant and ovulatory follicles occurred when BW and BCS were decreased during feed restriction of beef heifers (Rhodes et al., 1995). Similarly, reduced energy intake in postpartum beef cows (Perry et al., 1991; Stagg et al., 1995b) and negative energy balance in postpartum dairy cows (Lucy et al., 1991) were associated with decreased maximum diameter of

Figure 2. Least squares means (MSE = 4.63) and least squares regression lines for estradiol concentrations during the last ovulatory cycle (Cycle −2) and the subsequent anovulatory cycle (Cycle −1) before the onset of nutritionally induced anovulation. Treatment × cycle × day interaction (P < .001).
dominant follicles. In our study, the maximum diameter of the dominant follicle was less in R than in M heifers during Cycles −2 and −1 before R heifers ceased ovulations. However, maximum diameter of the dominant follicle and its growth rate within R heifers was not different between Cycle −2 (the last ovulatory) and Cycle −1 (the anovulatory cycle). Therefore, a reduction in diameter alone does not determine the ability of a dominant follicle to ovulate.

Concentration of estradiol in plasma of M and R heifers during Cycle −2 were similar, even though there was a substantial (34%) decrease in the diameter of the ovulatory follicle in R heifers compared with M heifers. Although the maximum diameter of the dominant follicle was similar during Cycles −2 and −1 within R heifers, concentrations of estradiol were less during Cycles −2 vs −1, and a preovulatory increase in estradiol did not occur during the anovulatory cycle. Rhodes et al. (1996) found a reduction in concentrations of estradiol in plasma of feed restricted beef heifers in the days following luteolysis during the last estrous cycle before anovulation, but concentrations of estradiol during the last ovulatory cycle were not reported. Prepubertal heifers that were adequately fed had greater concentrations of estradiol in plasma than did feed-restricted heifers, and concentrations were associated with diameter of dominant follicles (Bergfeld et al., 1994).

Concentration and pulse amplitude of LH were reduced in R heifers during Cycle −1 but not during Cycle −2, and pulse frequency of LH was reduced in R heifers during the late follicular phase compared with M heifers in both Cycles −2 and −1. Reduction in LH pulse frequency may result in decreased maximum diameter of the dominant follicle, and a reduction in LH concentrations and/or pulse amplitude may result in decreased estradiol concentrations and failure of ovulation. Suppression of pulsatile LH secretion in cattle with a GnRH agonist resulted in a substantial decrease in the maximum diameter of the dominant follicle (Gong et al., 1995). Reduction in diameter of dominant follicles was observed in feed-restricted postpartum beef cows compared with adequately fed cows, and this was associated with reduced LH pulse frequency (Perry et al., 1991). The relatively large increase in diameter of dominant follicles in the month preceding puberty (Bergfeld et al., 1994) in R heifers was associated with increased LH pulse frequency before puberty (Kinder et al., 1995).

Although LH secretion is reduced before the onset of nutritionally induced anovulation, FSH secretion increased during the late follicular phase of the anovulatory cycle (Cycle −1) in R heifers compared with M heifers, and this was associated with reduced concentrations of estradiol in plasma. Similarly, the onset of nutritionally induced anestrus in Bos indicus beef heifers was associated with increased concentrations of FSH in serum (Rhodes et al., 1996). However, Stagg et al. (1995a) found that concentrations of FSH were similar during normal estrous cycles, nutritionally induced anestrus, and resumption of cyclicity after realimentation of nutritionally anestrous heifers. Emergence of a follicular wave is preceded by a surge of FSH that is coincident with the cessation of growth of a dominant nonovulatory follicle or ovulation (Adams et al., 1992). Increased FSH concentrations preceding emergence of a follicular wave have been attributed to declining concentrations of inhibitory substances (estradiol, inhibin, or other proteins) originating from the dominant follicle of the previous wave (Ginther et al., 1996). During the anovulatory cycle in the present study (Cycle −1), arrest of the dominant follicle due to insufficient LH support in R heifers may have triggered the increased FSH concentrations and emergence of a new wave.

Feed restriction resulted in reduced maximum diameter of CL by 21 to 31% during the last two cycles before the onset of anovulation, and this was associated with reduced concentrations of progesterone in plasma. Various levels of feed restriction increased (McCann and Hansel, 1986), decreased (Imakawa et al., 1986; Villa-Godoy et al., 1990), or had no effect (Murphy et al., 1991; Rhodes et al., 1996) on peripheral concentrations of progesterone. Differences in severity of undernutrition and sampling periods may account for the differences in progesterone concentrations found in previous studies. Cows fed restricted diets had decreased CL weights (Rasby et al., 1991), and a linear decrease in maximum CL diameter was associated with decreasing body weight and condition (Rhodes et al., 1996). Because receptors for LH in bovine CL are not decreased with feed restriction (Schrick et al., 1992), reduced ovulatory size of follicles and pulse frequency of LH during feed deprivation are probably causes of reduced CL function.

Concentrations of NEFA in plasma are inversely related to feed intake or energy balance in ruminants (Lucy et al., 1991). Reduced feed intake in cows is associated with increased concentrations of NEFA (Richards et al., 1989b) as a result of increased lipolysis and fatty acid release from adipocytes. Concentrations of NEFA were greater in R than in M heifers before the onset of nutritionally induced anovulation in the present study. Concentrations of NEFA in plasma increased in feed restricted beef cows before changes in LH concentrations could be detected (Richards et al., 1989b), and short-term infusion of free fatty acids in ovariecetomized lambs did not alter pulsatile LH release (Estienne et al., 1990). However, the frequency of LH pulses was negatively correlated with concentrations of NEFA in plasma of primiparous, suckled cows (Grimard et al., 1995). In addition, increased NEFA concentrations may have a negative influence on ovarian function. Nonesterified fatty acids, and particularly oleic acid, negatively
affect LH-induced testosterone production in mouse Leydig cells in vitro by inhibiting cholesterol esterase and cholesterol utilization (Meikle et al., 1996). A similar role of NEFA on LH-induced androstenedione production by theca cells is possible. Concentrations of NEFA in R heifers during Cycle −1 were less than during Cycle −2; this is indicative of fat depletion or reduced metabolic rate in chronically underfed heifers. Feed restriction reduces resting metabolic rate in heifers and steers (Lapierre et al., 1992b; Yamabayama et al., 1996).

Increased NEFA concentrations in plasma of feed-restricted cycling heifers are associated with increased concentrations of GH (Armstrong et al., 1993). Concentration and pulse amplitude of GH were greater in R than in M heifers, but pulse frequency of GH was not affected by treatment. Restricted nutrient intake increases GH concentrations in sheep (Thomas et al., 1994), pigs, and cattle (Armstrong and Benoit, 1996). Increased GH concentrations during dietary restriction have been attributed to increased pulse amplitude (Breier et al., 1986; Yelich et al., 1996) or increased pulse frequency (Armstrong et al., 1993) of GH. Restricted feed intake in sheep did not alter concentrations, pulse frequency, and amplitude of GHRH in pituitary portal vessels, but concentrations of somatotropin release inhibiting factor were reduced by 50% compared with control animals (Thomas et al., 1994). Other factors such as reduced plasma IGF-I (Berelowitz et al., 1981) or decreased metabolic clearance of GH might contribute to increased concentrations of GH during feed deprivation (Trenkle, 1976; Lapierre et al., 1992a).

Despite increased GH secretion, concentrations of IGF-I were markedly reduced, indicating that the liver is insensitive to GH in undernourished animals. Peripheral concentrations of IGF-I and the response to intravenous injections of GH in cattle are reduced during periods of restricted protein and(or) energy intake (Breier et al., 1988; Armstrong et al., 1993). The cause of the reduced IGF-I response of the liver to GH during feed restriction may involve a decrease in hepatic GH receptors (Breier et al., 1988) as well as an uncoupling of the GH receptor from its intracellular response cascade (Thissen et al., 1991).

Growth hormone treatment stimulates follicular growth in cows (Lucy et al., 1993b), and GHRH treatment increased the size of large follicles in heifers (Spicer and Enright, 1991). Because exogenous GH or GHRH also increases IGF-I secretion, it cannot be determined whether these effects are due to GH or IGF-I. Although GH directly influences bovine granulosa cell function in vitro (Spicer and Stewart, 1996), the effects are minimal compared with those of IGF-I (Stewart et al., 1996). Even though GH receptors have been identified in follicles, the number is minimal (Lucy et al., 1993a). Thus, it is unlikely that the substantial increase in circulatory GH during underfeeding mediates effects of feed restriction on the ovary.

Substantial reduction in IGF-I concentrations during Cycle −2 in R heifers was not associated with reduced peripheral estradiol concentrations but was associated with reduced maximum diameter of the ovulatory follicles and maximum CL diameter. In agreement with our findings, Lucy et al. (1996) found that in miniature Brahman cows, which have low IGF-I concentrations due to a GH-receptor defect, the maximum diameter of dominant follicles and CL are reduced without any difference in peripheral concentrations of estradiol and LH concentrations. Short-term feed deprivation or chronic feed restriction do not affect intrafollicular concentrations of IGF-I in follicles that are less than 7 mm in diameter (Spicer et al., 1992; Kirby et al., 1993). The effect of underfeeding on concentrations of biologically active IGF-I in reproductive tissues needs further study because feed restriction alters concentrations of IGF binding proteins (Vandehaart et al., 1995).

In agreement with our results, feed restriction reduced plasma concentration of insulin in heifers (McCann and Hansel, 1986) and cows (Richards et al., 1989b; Grimard et al., 1995). Intracerebroventricular infusion of insulin in feed-restricted ovariotomized ewes increased secretion of LH within 24 h (Daniel et al., 1997). In contrast, intracerebroventricular infusion of insulin in growth-restricted ovariotomized ewes did not alter LH secretion (Hileman et al., 1993). In the present study, a 50% reduction in insulin concentrations in plasma of R heifers during Cycle −2 did not prevent ovulation. Concentrations of insulin in plasma of R heifers during Cycle −1 were similar to those during Cycle −2; however, reduced concentrations of LH and anovulation were observed. This observation indicates that changes in insulin concentrations during feed restriction may not provide the signal for the onset of nutritionally induced anovulation, or that several weeks may be required after the onset of an insulin deficiency before ovulation is prevented.

Physiological concentrations of insulin are probably required for normal follicular steroidogenesis. Granulosa cells of cattle have receptors for insulin (Spicer et al., 1994). Insulin is a potent stimulator of FSH-induced estradiol production by bovine granulosa cells (Spicer et al., 1994; Spicer and Stewart, 1996), and insulin infusion during a superovulatory regimen in cattle increased intrafollicular concentrations of estradiol in large follicles by fivefold (Simpson et al., 1994). A 50% reduction in peripheral insulin concentrations during Cycle −2 in the present study was not associated with reduced peripheral estradiol concentrations. Spicer et al. (1994) found that IGF-I inhibited insulin-induced estradiol production by granulosa cells from both small and large bovine follicles, indicating that IGF-I can act as an insulin...
antagonist. Thus, the seven- to tenfold reduction in peripheral IGF-I concentrations observed during Cycle –2 in R heifers may have counteracted the 40 to 50% reduction in peripheral insulin concentrations, resulting in unaltered estradiol concentrations in the last ovulatory cycle.

Chronic feed restriction in ruminants and loss in BW and (or) BCS are associated with decreased glucose concentrations (McCann and Hansel, 1986; Richards et al., 1989b). Glucose concentrations were less in R than in M heifers during the last two cycles before the onset of anovulation. These reduced concentrations of glucose probably had little impact on ovarian function directly, because in vitro studies indicate that, although glucose is needed for maximal steroidogenesis, glucose at concentrations of 25 to 75 mg/dL had similar stimulatory effects on steroidogenesis (Stewart et al., 1995). Changes in glucose concentrations may influence hypothalamic pituitary function. Injection of 2-deoxyglucose (2DG; inhibitor of glucose utilization) before or during the estrous cycle in cyclic beef heifers prevented estrus and CL formation (McClure et al., 1978). Systemic glucose infusion in lactating anestrous beef cows increased pulse frequency and concentrations of LH in serum during treatment with GnRH (Garmedia, 1986), and glucose concentrations were positively associated with feed intake and LH pulse frequency in prepubertal heifers fed at two levels of nutrition (Yelich et al., 1996). Intracerebroventricular infusion of 2DG to gonadectomized male lambs, at doses that did not affect peripheral concentrations of glucose, suppressed concentrations of LH in serum (Bucholtz et al., 1996). However, concentrations of glucose in plasma of postpartum cows are not predictive of luteal activity (Vizcarra et al., 1998). Glucose infusion in postpartum cows with good BCS and nonlactating well-fed cows did not alter LH secretion (McCaughey et al., 1988; Rutter et al., 1989), and infusion of 2DG in well-fed ewes had no effect on LH secretion (Hileman et al., 1991). It is possible that the effect of glucose on LH secretion depends on BCS of cattle and total energy availability.

Restriction of nutrient intake for an extended interval alters metabolism and endocrine function, and eventually the ovulatory follicle does not mature. Size of the dominant follicle is similar during the anovulatory cycle and the preceding cycle; however, both follicles are smaller than those in heifers maintaining body condition. Although concentrations of GH, IGF-I, NEFA, glucose, and insulin were altered during the last two cycles in heifers before anovulation compared with maintenance heifers, only concentrations of IGF-I and NEFA were different at the anovulation cycle compared with the preceding cycle in restricted heifers. We propose that IGF-I and NEFA may be two of many signals that influence hypothalamic secretion of GnRH, resulting in decreased secretion of LH during the anovulatory cycle. Reduced secretion of LH results in decreased production of estradiol by the dominant follicle and absence of ovulation. Further studies are needed to determine whether reduced follicular growth of chronically underfed but ovulatory heifers reduces fertilization rate and subsequent reproductive efficiency.

**Implications**

Long-term nutritional restriction that results in loss of body weight and body condition score (BCS) culminates with anovulation. Ovulatory and nonovulatory follicles in restricted heifers are similar in size but smaller than those in heifers maintaining BCS. Although there is not an adequate predictor that estrous cycles will be anovulatory due to nutritional restriction, a “thin” BCS and altered plasma concentrations of glucose, insulin, insulin-like growth factor-I, nonesterified fatty acids, and growth hormone in restricted heifers, compared with maintenance heifers, are indicators that continued nutritional restriction may result in the cessation of ovulatory cycles.

**Literature Cited**


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