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# Nutritionally Induced Anovulation in Beef Heifers: Ovarian and Endocrine Function During Realimentation and Resumption of Ovulation[1]

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## ABSTRACT

**Nutritionally induced anovulatory and cyclic Angus × Hereford heifers were used to evaluate follicular growth and concentrations of hormones and metabolites during anovulation and resumption of ovulation. Anovulatory heifers were fed to gain 0.6 (LGAIN) or 1.5 (HGAIN) kg/day until resumption of ovulation, and heifers with normal estrous cycles were fed a maintenance diet (M). Follicles ≥ 4 mm in diameter were measured by daily ultrasonography in HGAIN and LGAIN heifers during one follicular wave before realimentation (Wan) and in two waves (W-2, W-1) immediately before the wave resulting in first ovulation or luteinization (W0). Ovaries of M heifers were evaluated to determine the day of ovulation of the second-wave dominant follicle (DF). Resumption of ovulation after realimentation occurred 23 days earlier in HGAIN than in LGAIN. Maximum diameter, growth rate, and persistence of dominant follicles increased, while persistence of first subordinate follicles decreased between anovulation and resumption of ovulation in anovulatory heifers. Concentrations of LH in serum were similar for HGAIN and LGAIN and gradually increased during realimentation. The increase in estradiol before the first ovulation was less in realimented heifers compared with cyclic heifers. Concentrations of insulin-like growth factor-I (IGF-I) in HGAIN and LGAIN gradually increased during realimentation but were lower than concentrations of IGF-I in cyclic heifers at ovulation. Increased diameter, growth rate, and persistence of the DF were associated with increased concentrations of LH, estradiol, and IGF-I during the transition from nutritionally induced anovulation to resumption of ovulatory cycles.**

## INTRODUCTION

Reproductive performance of beef cows is associated with body condition score (BCS) [1–3]. Body condition at calving influences the duration of the postpartum anestrous period [4–6], and increases in body weight and BCS are required for resumption of estrous cycles after nutritionally induced anestrus [7].

Reduced energy intake delays the development of dominant follicles in prepubertal heifers [8] and postpartum cows [9]. A linear increase in persistence, growth rate, and maximum size of dominant follicles occurs during realimentation of nutritionally anestrous beef heifers [10].

The mechanisms whereby undernutrition causes anestrus, and realimentation results in resumption of cyclicity in cattle, have not been determined. Feed restriction suppresses secretion of LH in beef cows [7, 11], cyclic heifers [12], and prepubertal heifers [13, 14]; and this effect is probably mediated by reduced GnRH secretion, since exogenous administration of GnRH to nutritionally anestrous cows induces luteal activity [15, 16]. Alterations in concentrations of growth hormone, insulin, insulin-like growth factor-I (IGF-I), glucose, and nonesterified fatty acids (NEFA) in blood are indicative of energy availability and may provide short- or long-term signals that mediate the effects of nutrition on LH secretion. Follicular growth, gonadotropins in serum, and concentrations of metabolic hormones and compounds in blood at particular stages of follicular waves during nutritionally induced anovulation and resumption of ovulation after realimentation have not been documented. The objectives of this experiment were to evaluate the effect of two rates of gain during realimentation on time, body weight, and BCS at first ovulation after nutritionally induced anovulation and to evaluate follicular growth and concentration of LH, FSH, estradiol, IGF-I, insulin, glucose, and NEFA in blood during the transition from nutritionally induced anovulation to resumption of ovulation.

## MATERIALS AND METHODS

### *Animals and Procedures*

Twelve nutritionally induced anovulatory beef heifers with a body weight of 298 ± 3 kg and a BCS (1 = emaciated; 9 = obese) [17] of 3.8 ± 0.1, and six cyclic heifers with a body weight of 453 ± 10 kg and a BCS of 5.2 ± 0.2, were used in two replications to determine follicular growth and concentrations of hormones and metabolites during anovulation and resumption of ovulatory cycles. Heifers were of similar age (28–30 mo) at realimentation, and differences in body weight and BCS were the result of a nutritional regimen to induce anovulation. Diets were restricted so heifers lost 0.70 ± 0.03% of their body weight per week, and anovulation occurred when heifers had lost 22 ± 2% of their body weight. Follicular and endocrine changes in these heifers during the onset of anovulation have been reported [11]. Commencing at approximately 6 wk after the onset of anovulation, ultrasonography was performed and plasma and serum samples were collected daily from heifers until a complete follicular wave occurred. Twenty-two days were required to obtain at least one follicular wave for all anovulatory heifers. During anovulation, heifers were fed 5 kg of prairie hay per day. Thereafter, heifers were randomly assigned to one of two groups and fed a complete diet (Table 1) to gain 0.6 (LGAIN) or 1.5 (HGAIN) kg/day. At the initiation of realimentation, 10 days were required to gradually increase the amount of diet for each group. The amount of feed provided each day was adjusted every second week to maintain the prescribed rate of gain. Shrunk body weight (after 16-h withdrawal of feed and water) was obtained weekly and BCS every 2 wk. One

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TABLE 1. Composition of diets fed to heifers for high gain (HGAIN), low gain (LGAIN) and to maintain (M) body condition.

| Item | HGAIN | LGAIN | M |
|---|---|---|---|
| Ingredients, as fed, % | | | |
| Corn distillers gain | 23.2 | 20.0 | — |
| Rolled corn | 38.2 | 32.9 | 37.5 |
| Cottonseed hulls | 7.7 | 6.7 | 21.7 |
| Alfalfa pellets | 3.9 | 3.4 | 32.5 |
| Prairie hay | 22.7 | 33.3 | — |
| Cane molasses | 3.5 | 3.0 | 3.0 |
| Soybean meal | — | — | 5.0 |
| Limestone 38% | 0.5 | 0.5 | — |
| Salt | 0.2 | 0.2 | 0.3 |
| Zinc oxide | 0.002 | 0.002 | — |
| Vitamin A, 30,000 | 0.02 | 0.02 | — |
| Vitamin E, 50% | 0.02 | 0.02 | — |
| Calculated values as fed | | | |
| Kg | 10.0 | 6.8 | 4.5 |
| DM % | 89.7 | 86.6 | 88.9 |
| Total NEm, Mcal | 16.2 | 10.2 | 6.7 |
| Total NEg, Mcal | 9.7 | 6.1 | 3.6 |
| Crude Protein % | 10.4 | 9.6 | 12.2 |

heifer of the HGAIN group was removed from the trial after development of leg defects. During realimentation, ultrasonography was performed and plasma and serum samples were collected daily until ovulation resulting in a normal estrous cycle (>17 days). Cyclic heifers (M) were fed a diet to maintain BCS (Table 1), and estrous cycles were synchronized by treatment with prostaglandin $F_{2\alpha}$ (25 mg of Lutalyse; Pharmacia and Upjohn, Kalamazoo, MI) followed by a second treatment 11 days later. Starting on Day 13 of the induced cycle (Day 1: day of ovulation), M heifers were given prostaglandin $F_{2\alpha}$ every 16 days thereafter to synchronize and maintain 16-day estrous cycles until realimented heifers resumed ovulations. Ovaries of cyclic heifers were evaluated (at coincident times as anovulatory heifers) by ultrasonography, and serum and plasma samples were collected daily from Day 8 until the subsequent ovulation. All experimental procedures were approved by the Oklahoma State University Animal Care and Use committee.

### *Ultrasonography and Follicular Measurements*

Transrectal ultrasonography was performed with an Aloka 500V ultrasound scanner equipped with a 7.5-MHz transducer (Corometrics Medical Systems, Wallingford, CT). During ultrasound scanning, approximate position and size of follicles and corpora lutea (CL) in both ovaries were sketched. Scans of the ovaries were also recorded on a videotape and viewed later to draw complete ovarian maps recording all follicles ≥ 4 mm. Reference points on the ovaries included the poles and the hilus [18]. Size of follicles was calculated as the mean of the longest and shortest diameters. Follicles ≥ 4 mm in diameter were sequentially identified and measured in one follicular wave before realimentation (Wan), two waves (W-2, W-1) immediately before the first wave resulting in ovulation or luteinization (W0), and during W0. A dominant follicle (DF) was identified as luteinized when luteal tissue (thickened wall) surrounding the nonechogenic area (dark) of a follicle developed without previous ovulation [19]. For each follicular wave, diameters of the DF and first subordinate (SF) follicles were determined. Changes in diameter of DF and SF were used to determine growing, static, and regressing phases [20] so that comparisons could be made among waves. Day of emergence of the DF was defined as the day before the first day that the ovulatory follicle could be individually identified. Growing phase was defined as the interval between the day of emergence and the day that the follicle ceased its progressive increase in diameter by 0.5 mm or more. Growth rate of the DF was estimated as the increase in diameter from the day of emergence to the maximum diameter divided by days of growth. Static phase was defined as the interval between the last day of the growing phase and the first day that the follicle began a progressive decrease in diameter by 0.5 mm or more, and was characterized by the number of days and the average diameter during the static phase. The regression phase was the interval from the last day of the static phase until the last day that the follicle could be identified on the ovary, and was characterized by the regression rate in mm/day and duration of the phase.

### *Collection of Blood Samples and Hormone Analyses*

Concentrations of LH and FSH in serum and estradiol-17β, insulin, IGF-I, glucose, and NEFA in plasma were determined in samples during the last 5 days of the growing phase of the DF in anovulatory heifers and the ovulatory follicle in cyclic heifers at four periods: Wan, W-2, W-1, W0, and during W0 in anovulatory heifers. Serum and plasma samples were obtained at coincident times in cyclic heifers. Blood samples were collected via tail venipuncture. For serum, samples were allowed to clot for 24 h at 4°C and then centrifuged at 2800 × *g* for 20 min. Plasma was obtained from blood collected in 15-ml tubes containing EDTA (0.1 ml of a 15% solution) and placed on ice and centrifuged within 1 h at 2800 × *g* for 20 min. Estradiol-17β concentrations in plasma were quantified by an RIA (estradiol MAIA; Polymedco, New York, NY) with modifications [16]. Intra- and interassay coefficients of variation (n = 4 assays) were 10% and 14%, respectively. Concentrations of LH in serum were quantified by RIA [15], with NIH LH-B9 (Bethedsa, MD) as the standard, and intra- and interassay coefficients of variation (n = 2 assays) were 9% and 15%, respectively. Concentrations of FSH in serum were quantified by RIA [16], with USDA-bFSH-I-2 as the standard, and intra- and interassay coefficients of variation (n = 2 assays) were 3% and 7%, respectively. Concentrations of IGF-I in plasma were quantified by RIA [21] after an acid ethanol extraction (16 h at 4°C). Recombinant human IGF-I (R&D Systems, Minneapolis, MN) was used as standard, and intra- and interassay coefficients of variation (n = 2 assays) were 3% and 16%, respectively. Concentrations of insulin in plasma were quantified by a solid-phase RIA for human insulin (Coat-A-Count insulin kit; Diagnostic Products, Los Angeles, CA) using bovine pancreatic insulin as the standard (28.6 USP U/mg; Sigma Chemical Co., St. Louis, MO) and 0.2-ml sample volume. Sensitivity of the assay was 0.05 ng/ml plasma; and addition of 0.8, 1.6, and 3.2 ng of insulin in 1 ml of plasma resulted in recovery of 97%, 109%, and 108%, respectively (n = 4). When 0.05, 0.10, 0.15, and 0.20 ml of plasma were assayed, concentrations of insulin were parallel to the standard curve. Intra- and interassay coefficients of variation (n = 2 assays) were 4% and 9%, respectively. Concentrations of glucose in plasma were determined by an enzymatic colorimetric procedure (Sigma, no. 510). Intra- and interassay coefficients of variation (n = 2 assays) were 4% and 14%, respectively. Concentrations of NEFA in plasma were determined by an enzymatic colorimetric procedure (Wako-NEFA C; Wako Chemicals, Dallas, TX) with modification

TABLE 2. Influence of daily gain during realimentation of nutritionally anovulatory heifers[a] fed at two different levels of nutrition on interval between anovulation and resumption of ovulation and body weight and BCS at first ovulation or luteinization.

| Parameter | HGAIN | LGAIN | MSE |
|---|---|---|---|
| Days to first ovulation or luteinization after realimentation, days | 57[b] | 80[c] | 75 |
| Body weight at anovulation, kg | 295 | 300 | 117 |
| Body weight at first ovulation or luteinization, kg | 387 | 343 | 94 |
| Change in BW from anovulation to resumption of ovulation, % | 28[b] | 16[c] | 10 |
| BCS at anovulation | 3.8 | 3.8 | 0.1 |
| BCS at first ovulation or luteinization | 4.7 | 4.4 | 0.2 |

[a] Anovulation was determined by regression of the dominant follicle and absence of a corpus luteum with progesterone in plasma less than 0.5 ng/ml at 7 d after the expected time of ovulation and during the subsequent 3 wk.
[b,c] Means within a row lacking a common superscript differ ($P < 0.05$).

[22]. Intra- and interassay coefficients of variation (n = 2 assays) were 6% and 11%, respectively.

### *Statistical Analysis*

The interval between anovulation and resumption of ovulation, and body weight and BCS at first ovulation, were compared between HGAIN and LGAIN by ANOVA in a 2 × 2 factorial with replication (rep) and treatment (HGAIN and LGAIN).

Follicular wave parameters were compared among reps, treatments (HGAIN and LGAIN), and waves using a split-plot ANOVA with rep, treatment, and treatment × rep in the main plot and wave, rep × wave, treatment × wave, and treatment × wave × rep in the subplot. Follicular measurements for the M heifers were not included in the analysis due to the use of an estrous synchronization regimen in these animals. Mean square error (MSE) of heifer within treatment × rep was used as the error term for the main plot effects.

Multivariate ANOVAs for repeated measures were used to determine the effect of treatment (M, HGAIN, LGAIN), wave (Wan, W-2, W-1, and W0), and Day (0, −1, −2, −3, −4, where Day 0 is the day that the DF had a maximum diameter in anovulatory heifers [HGAIN and LGAIN] and 1 day before ovulation in cyclic [M] heifers) on LH, FSH, estradiol, IGF-I, insulin, glucose, and NEFA concentrations. Concentrations of hormones and metabolites during days represented the repeated response variable (within-subject factors). The between-subject factors were treatment, rep, and treatment × rep in the main plot and wave, treatment × wave, rep × wave, and treatment × rep × wave in the subplot. Mean square error of heifer within treatment × rep was the error term for the factors in the main plot. The residual MSE was the error term for the factors in the subplot. Because interactions of treatment and wave with rep either were not significant or were due to differences in the magnitude of the response and not in direction, data for the two replications were combined and rep was removed from the model. If interactions with day were significant, polynomial response curves of appropriate order were fit and tested for homogeneity of regression [23]. Tukey-Kramer's test [24] for pair-wise comparisons was used to compare means among treatments and waves.

## RESULTS

### *Follicular Data*

Increased nutrient intake of nutritionally anovulatory beef heifers resulted in increased body weight and BCS and resumption of ovarian cycles (Table 2). During W0, nine heifers ovulated and two developed a luteinized follicle. The interval between initiation of realimentation and first ovulation or luteinization was shorter for HGAIN than for LGAIN ($P < 0.05$). At first ovulation or luteinization, BCS was not different ($P > 0.10$) between HGAIN and LGAIN; however, body weight was greater ($P < 0.05$) for HGAIN compared with LGAIN.

Neither treatment (HGAIN and LGAIN) nor treatment × wave influenced follicular characteristics, so data for the treatments were combined. Wave influenced ($P < 0.0001$) the maximum diameter of DF, and size increased from anovulation to first ovulation or luteinization (Table 3). Wave also influenced growth rate of DF ($P < 0.0001$), which increased after realimentation, but was not different between W-1 and W0. Duration of the growing phase of DF was greater ($P < 0.05$) in W-2, W-1, and W0 compared with Wan, and duration of the static phase of DF was greater ($P < 0.05$) in W-2 and W-1 compared with Wan.

Static phase of the DF was 2.5 ± 0.3 days before ovulation or luteinization during W0. A static phase did not occur in cycling heifers before ovulations. Regression rate of DF increased ($P < 0.001$) between W-2 and W-1 during realimentation. Duration of the regression phase of DF was greater ($P < 0.05$) in W-2 and W-1 compared with Wan.

Wave influenced ($P < 0.0001$) growth and regression rates of SF and duration of growing, static, and regression phases. Growth and regression rates of first SF gradually increased while the duration of growing, static, and regression phases gradually decreased in successive waves during realimentation (Table 4). The maximum diameter of first SF during W-2, W-1, and W0 was greater ($P < 0.05$) than the maximum diameter of first SF during Wan.

During W0, nine heifers ovulated and two developed a luteinized follicle. All nine heifers that ovulated had a short

TABLE 3. Characteristics of dominant follicles during waves at anovulation before realimentation (Wan), and at two waves (W-2, W-1) immediately before ovulation or luteinization (W0) in HGAIN and LGAIN heifers.

| | Wave | | | | |
|---|---|---|---|---|---|
| Parameter | Wan | W-2 | W-1 | W0 | MSE |
| Growth rate, mm/days | 0.9[a] | 1.2[b] | 1.5[c] | 1.6[c] | 0.04 |
| Duration of growing phase, days | 5.2[a] | 6.4[b] | 6.4[b] | 7.0[b] | 0.7 |
| Max diameter, mm | 9.2[a] | 11.7[b] | 13.2[c] | 15.3[d] | 1.1 |
| Duration of static phase, days | 3.8[a] | 4.9[b] | 5.0[b] | 2.5[c] | 0.5 |
| Regression rate, mm/days | 1.0[a] | 1.2[b] | 1.5[c] | — | 0.02 |
| Duration of regression phase, days | 5.0[a] | 6.0[b] | 5.9[b] | — | 0.4 |
| Wave persistence, days | 14.0[a] | 17.2[b] | 17.4[b] | — | 4.8 |

[a,b,c,d] Means within a row lacking a common superscript differ ($P < 0.05$).

TABLE 4. Characteristics of subordinate follicles during waves at anovulation before realimentation (Wan), and at two waves (W-2, W-1) immediately before ovulation or luteinization (W0) in HGAIN and LGAIN heifers.

| Criteria | Wave | | | | MSE |
|---|---|---|---|---|---|
| | Wan | W-2 | W-1 | W0 | |
| Growth rate, mm/day | 0.8[a] | 1.2[b] | 1.4[c] | 1.7[c] | 0.02 |
| Duration of growing phase, days | 4.0[a] | 3.3[b] | 2.9[b] | 2.4[b] | 0.3 |
| Max diameter, mm | 7.3[a] | 8.3[b] | 8.2[b] | 8.2[b] | 0.6 |
| Duration of static phase, days | 2.9[a] | 2.4[b] | 2.0[b] | 1.4[c] | 0.08 |
| Regression rate, mm/days | 0.7[a] | 1.1[b] | 1.4[c] | 1.8[d] | 0.03 |
| Duration of regression phase, days | 4.3[a] | 3.6[b] | 2.8[c] | 2.2[d] | 0.2 |
| Wave persistence, days | 11.2[a] | 9.3[b] | 7.7[c] | 6.0[d] | 1.9 |

[a,b,c,d] Means within a row lacking a common superscript differ ($P < 0.05$).

cycle with an interovulatory interval of 10.5 ± 0.9 days. The other two heifers (one from the HGAIN and one from the LGAIN group) ovulated 9 and 10 days after the luteinized follicle was first detected. Concentrations of progesterone in plasma on Day 3, 4, 5, 6, 7, 8, and 9 after ovulation and luteinization averaged 0.2, 0.9, 1.6, 2.7, 1.1, 0.8, 0.4, and 0.6, 1.3, 1.5, 0.9, 0.6, 0.2, 0.1 ng/ml, respectively. The subsequent interovulatory interval was of normal duration in all heifers and averaged 20.2 ± 0.4 days.

### *Hormones and Energy Metabolites*

There was a treatment × wave × day effect ($P < 0.01$) on LH concentrations. Concentrations were best described by quadratic equations (Fig. 1). Analyses of homogeneity of regression indicated that M heifers had greater ($P < 0.05$) concentrations of LH than HGAIN and LGAIN during Wan, W-2, and W-1. Concentrations of LH were not different between HGAIN and LGAIN in any of the follicular waves ($P > 0.1$), and concentrations gradually increased after realimentation for both treatments. During W0, concentrations of LH were similar for M, HGAIN, and LGAIN.

Concentrations of FSH were not influenced ($P > 0.1$) by treatment or wave, but there was a day effect ($P < 0.0001$). Concentrations of FSH were best described by quadratic equations (Fig. 2) and increased during the last 5 days of the growing phase of DF.

There was a treatment × wave × day effect ($P < 0.05$) for concentrations of estradiol, and concentrations were best described by quadratic equations (Fig. 3). Analyses of homogeneity of regression indicated that M had greater ($P < 0.05$) estradiol concentrations than HGAIN and LGAIN

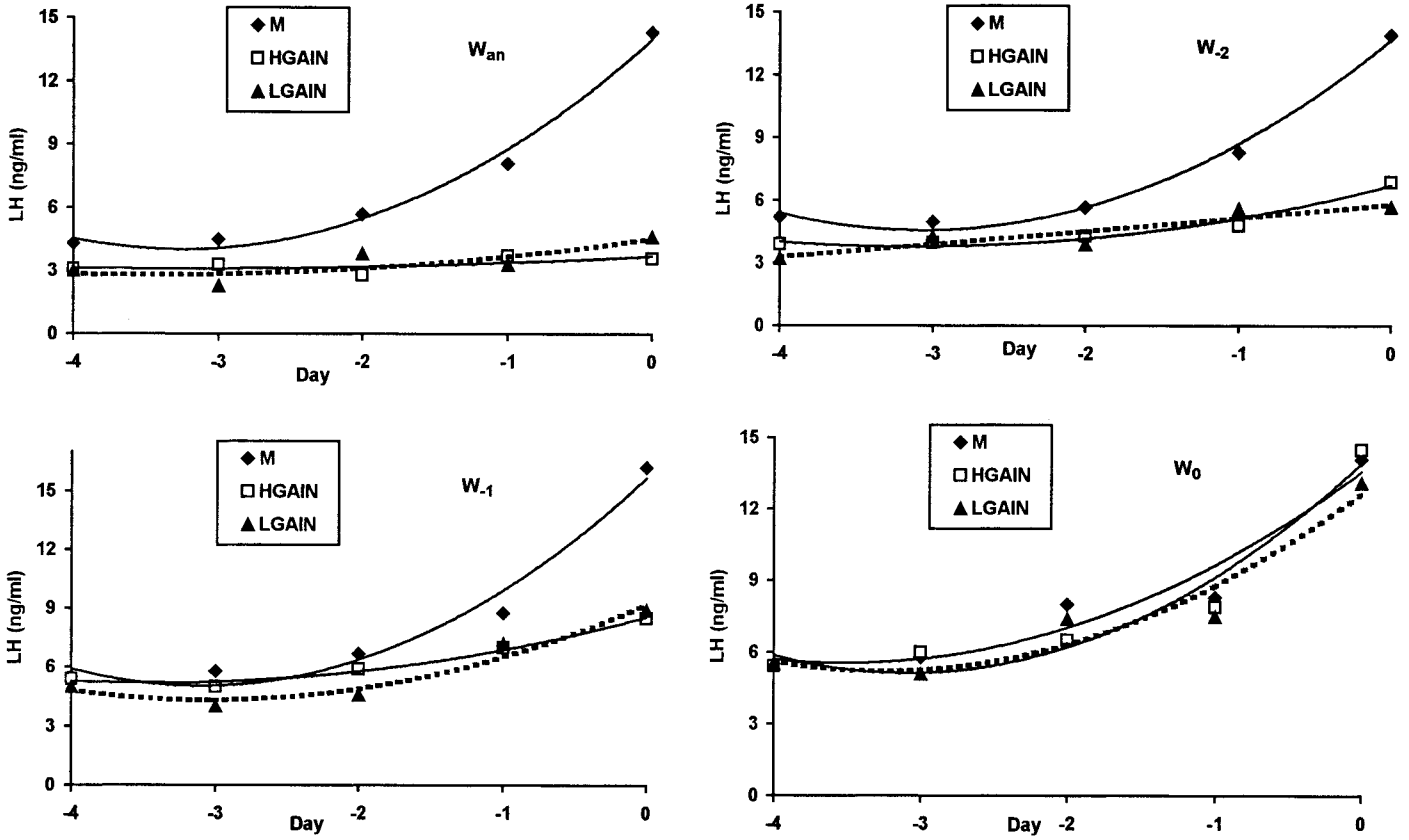


FIG. 1. Least square means (symbols) and least square regressions (lines) for concentrations of LH in cyclic heifers (M) or LGAIN or HGAIN heifers during follicular waves when heifers were anovulatory and before initiation of refeeding (Wan), two waves (W-2), or one wave (W-1) before ovulation or luteinization (W0) and W0. Treatment × wave × day effect ($P < 0.01$; MSE = 5.46), where Day 0 is the day that the DF had a maximum diameter in anovulatory heifers and 1 day before ovulation in cyclic heifers.

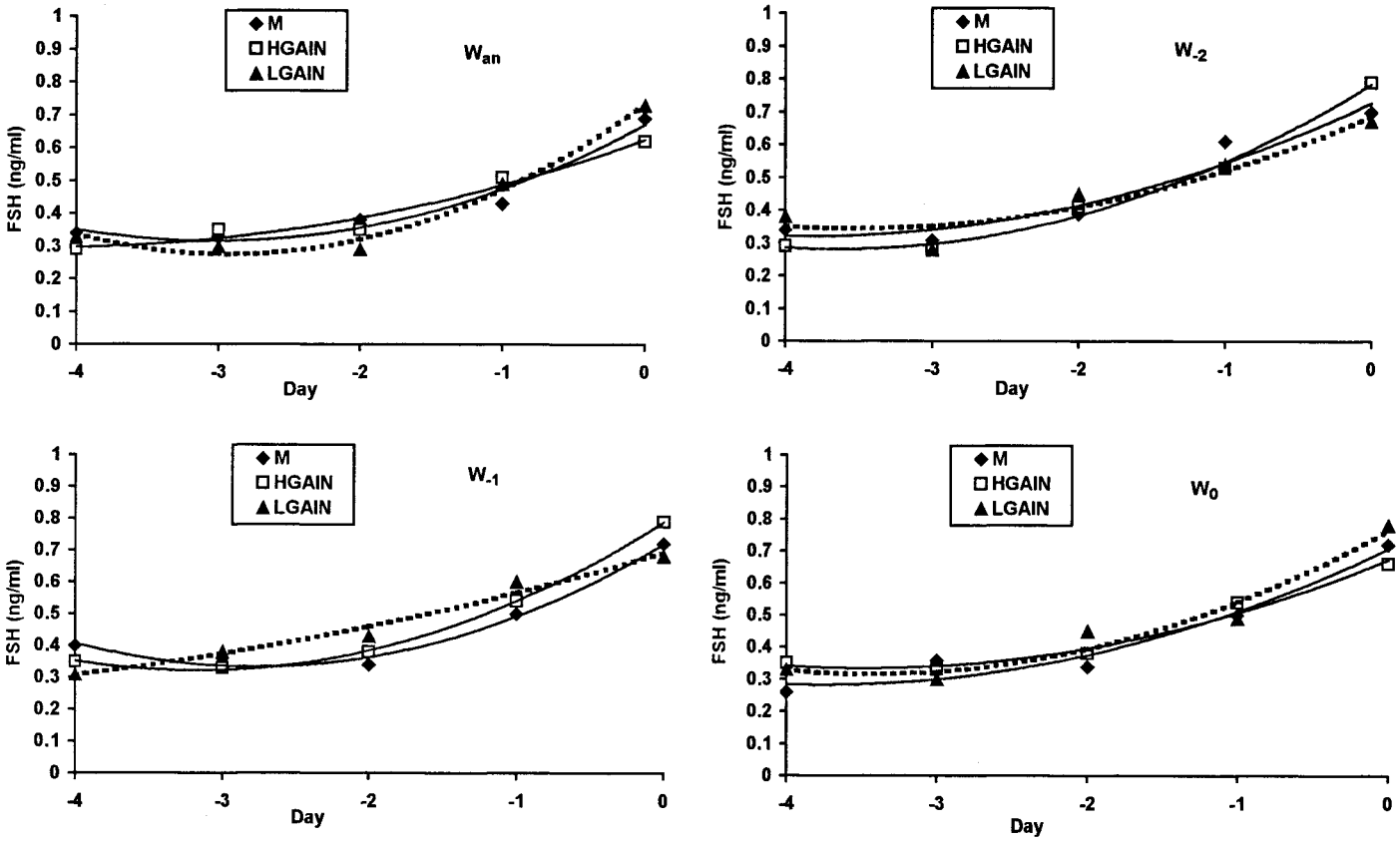


FIG. 2. Least square means (symbols) and least square regressions (lines) for concentrations of FSH in cyclic heifers (M) or LGAIN or HGAIN heifers during follicular waves when heifers were anovulatory and before initiation of refeeding (Wan), two waves (W-2), or one wave (W-1) before ovulation or luteinization (W0) and W0. Day effect ($P < 0.01$; MSE = 5.46), where Day 0 is the day that the DF had a maximum diameter in anovulatory heifers and 1 day before ovulation in cyclic heifers.

during all waves, and concentrations were similar in HGAIN and LGAIN. A gradual increase in estradiol concentrations occurred after realimentation in HGAIN and LGAIN. The magnitude of the preovulatory increase of estradiol during W0 was less in HGAIN and LGAIN compared with M. The maximum preovulatory increase of estradiol was coincident with the end of the growing phase. During W0, the DF in realimented heifers ovulated or luteinized approximately 3 days after the maximum preovulatory increase of estradiol occurred, whereas cycling heifers ovulated 1 day after the maximum preovulatory increase of estradiol occurred (data not shown).

Concentrations of IGF-I, glucose, insulin, and NEFA were not influenced by day; there were no interactions with day, so concentrations within treatments and waves were averaged over days. Concentrations of IGF-I were not different ($P > 0.1$) between HGAIN and LGAIN. There was a treatment × wave effect ($P < 0.0001$) on IGF-I concentrations. Concentrations of IGF-I in HGAIN and LGAIN gradually increased after realimentation but were less ($P < 0.05$) than concentrations of IGF-I in M heifers during all waves (Fig. 4).

There was a treatment × wave effect ($P < 0.01$) on glucose and insulin concentrations (Figs. 5 and 6). Concentrations of glucose and insulin were similar for HGAIN and LGAIN. Concentrations of glucose and insulin in HGAIN and LGAIN increased after realimentation, and concentrations were similar for M, HGAIN, and LGAIN during W-2, W-1, and W0.

Concentrations of NEFA were similar in HGAIN and LGAIN. There was a treatment × wave effect ($P < 0.0001$) on NEFA concentrations (Fig. 7). HGAIN and LGAIN had greater ($P < 0.05$) NEFA concentrations during Wan than M heifers. After realimentation, NEFA concentrations were less ($P < 0.05$) in HGAIN and LGAIN compared with M heifers; however, concentrations of NEFA gradually increased during W-2, W-1, and W0 in both HGAIN and LGAIN.

## DISCUSSION

Rate of gain during realimentation (HGAIN vs. LGAIN) had no effect on any of the follicular characteristics during the wave when ovulation occurred or the two preceding waves. However, increased average daily gain in HGAIN resulted in a 23-day-shorter interval between anovulation and resumption of ovulation after realimentation compared with that for LGAIN. Similarly, prepubertal beef heifers fed a low-energy diet had delayed puberty compared with heifers fed a high-energy diet, but follicular characteristics were similar for the two groups at 30, 60, 90, and 120 days before puberty [8]. Follicular parameters were not affected by energy intake of postpartum beef cows, even though cows fed the high-energy diet had a shorter postpartum interval to ovulation [9]. Realimentation of nutritionally induced anovulatory heifers in the present study resulted in a gradual increase in the maximum size, growth rate, regression rate, and persistence of dominant follicles. Reduced nutrient intake in cyclic [25] and prepubertal beef heifers [8] decreased persistence and maximum size of

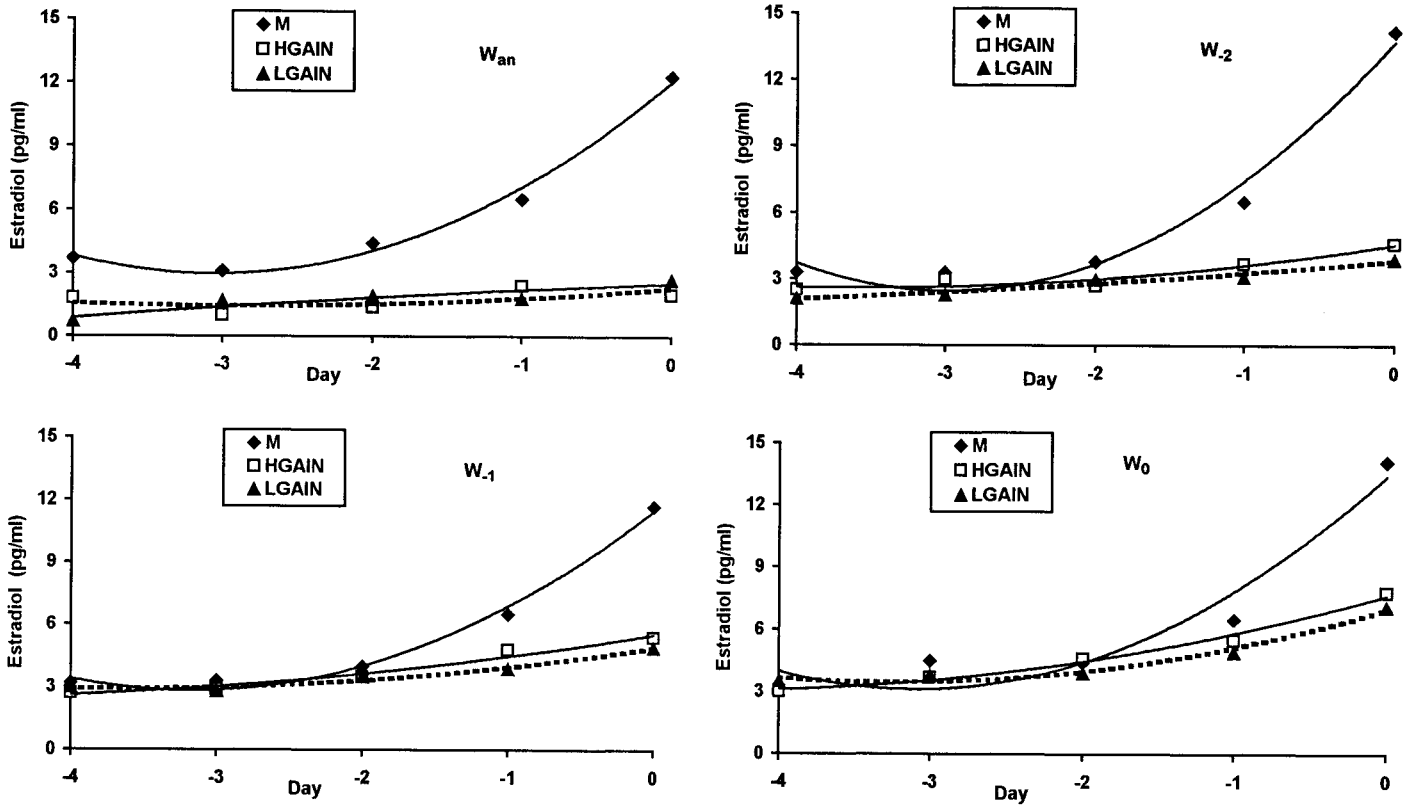


FIG. 3. Least square means (symbols) and least square regressions (lines) for concentrations of estradiol in cyclic heifers (M) or LGAIN or HGAIN heifers during follicular waves when heifers were anovulatory and before initiation of refeeding (Wan), two waves (W-2), or one (W-1) wave before ovulation or luteinization (W0) and W0. Treatment × wave × day effect ($P < 0.05$; MSE = 3.57), where Day 0 is the day that the DF had a maximum diameter in anovulatory heifers and 1 day before ovulation in cyclic heifers.

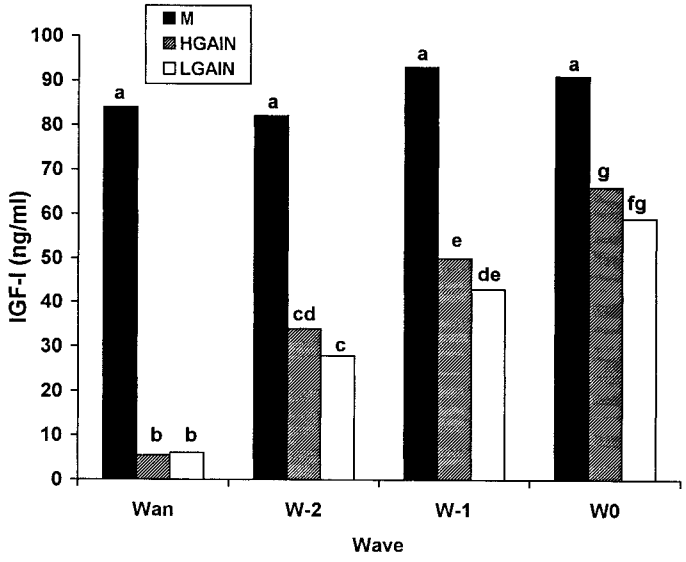


FIG. 4. Least square means for concentrations of IGF-I in cyclic heifers (M) or LGAIN or HGAIN heifers during follicular waves when heifers were anovulatory and before initiation of realimentation (Wan), two waves (W-2), or one wave (W-1) before ovulation or luteinization (W0) and W0. Concentrations for days (−4, −3, −2, −1, and 0) were averaged over treatment and wave. Treatment × wave ($P < 0.0001$; MSE = 153). Bars without a common superscript differ ($P < 0.05$).

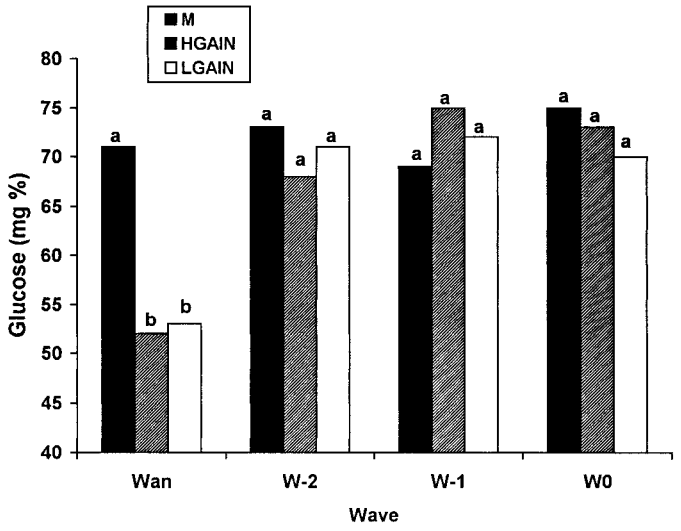


FIG. 5. Least square means for concentrations of glucose in cyclic heifers (M) LGAIN or HGAIN heifers during follicular waves when heifers were anovulatory and before initiation of realimentation (Wan), two waves (W-2), or one (W-1) wave before ovulation or luteinization (W0) and W0. Concentrations for days (−4, −3, −2, −1, and 0) were averaged over treatment and wave. Treatment × wave ($P < 0.01$; MSE = 81). Bars without a common superscript differ ($P < 0.05$).

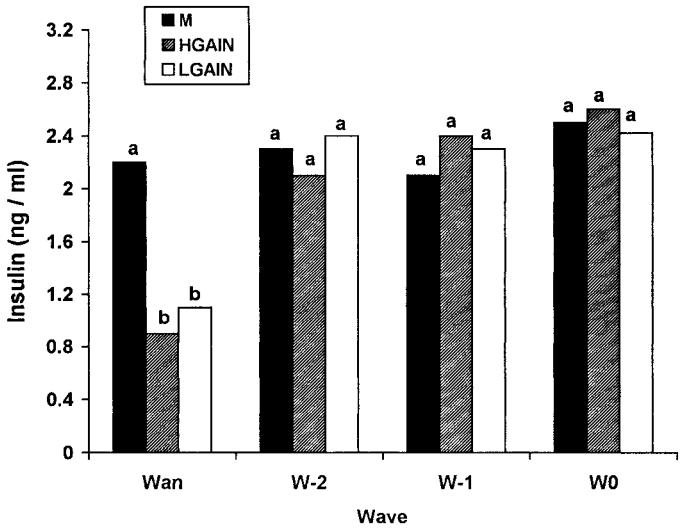


FIG. 6. Least square means for concentrations of insulin in cyclic heifers (M) or LGAIN or HGAIN heifers during follicular waves when heifers were anovulatory and before initiation of realimentation (Wan), two waves (W-2), or one (W-1) wave before ovulation or luteinization (W0) and W0. Concentrations for days (−4, −3, −2, −1, and 0) were averaged over treatment and wave. Treatment × wave ($P < 0.01$; MSE = 0.34). Bars without a common super script differ ($P < 0.05$).

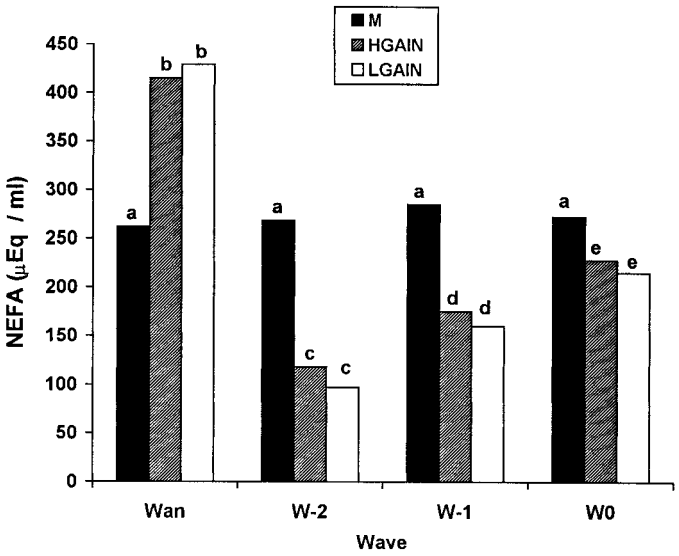


FIG. 7. Least square means for concentrations of NEFA in cyclic heifers (M) or LGAIN or HGAIN heifers during follicular waves when heifers were anovulatory and before initiation of realimentation (Wan), two waves (W-2), or one (W-1) wave before ovulation or luteinization (W0) and W0. Concentrations for days (−4, −3, −2, −1, and 0) were averaged over treatment and wave. Treatment × wave ($P < 0.0001$; MSE = 864). Bars without a common superscript differ ($P < 0.05$).

dominant follicles and tended to increase the incidence of three-wave cycles in cyclic heifers [25]. A linear reduction in persistence and maximum size of dominant and ovulatory follicles with decreasing body weight and condition score was observed in feed-restricted beef heifers, whereas a linear increase in persistence, growth rate, and maximum size of dominant follicles was observed during realimentation of nutritionally anestrous beef heifers [10]. Size of the ovulatory follicle was reduced in the heifers in this experiment when they were losing weight before anovulation; rate of growth of the DF was reduced during the cycle before the onset of anovulation and in the preceding cycle [11]. These findings indicate that decreased BCS, body weight, and/or feed intake in cattle results in reduced growth and persistence of dominant follicles; and increased feed intake, body weight, and BCS of undernourished cattle results in increased growth and persistence of dominant follicles.

In contrast with findings for dominant follicles, persistence of largest subordinate follicles was substantially decreased after realimentation in the present study. Reduced energy intake in postpartum beef cows reduces the size of dominant follicles [26] and the number of large estrogen-active follicles [27]. The persistence of small subordinate follicles is increased by reduced energy intake in beef cows [27]. Negative energy balance in postpartum dairy cows was associated with increased number of medium-sized follicles and decreased maximum diameter of dominant follicles, while positive energy balance was associated with increased maximum diameter of dominant follicles and reduced growth of subordinates [28]. These studies and our results indicate that reduced energy intake, body weight, and/or BCS increases persistence of subordinate follicles while increased energy intake, body weight, and/or BCS of underfed cattle decreases persistence of subordinate follicles.

The gradual increase in growth rate, size, and persistence of dominant follicles after realimentation was associated with increased concentrations of LH and IGF-I in serum. The importance of LH for maturation of ovulatory follicles in cattle has been reviewed [29]. Realimentation of nutritionally anestrous beef heifers resulted in resumption of cyclicity after 50 days of initiation of refeeding; and during this period pulse frequency, pulse amplitude, and mean concentrations of LH gradually increased [30]. The gradual increase in LH concentrations after realimentation, and before resumption of ovulation, was associated with a gradual increase in estradiol production. Persistence of dominant follicles and ability to produce sufficient amounts of estradiol to induce preovulatory surges of LH depend on secretion of LH [31]. We hypothesize that the GnRH pulse generator returns to normal function during realimentation of nutritionally anestrous heifers and that this allows a gradual increase in LH secretion that in turn increases ovarian output of estradiol and eventually resumption of ovulation. However, the delayed ovulation after the reduced preovulatory increase in estradiol in realimentated heifers indicates that ovulatory events were not completely normal.

Peripheral concentrations of IGF-I are positively associated with body condition and nutrient intake [13, 14, 32], and decreased concentrations of IGF-I are associated with delayed puberty [33] and increased postpartum anestrous intervals [34–36] in beef cattle. During the estrous cycle before these nutritionally restricted heifers became anovulatory, concentrations of IGF-I in plasma were only 12% of the concentrations in M heifers [11]. Changes in systemic IGF-I concentrations may directly impact ovarian function in cattle (for reviews see [37, 38]). Specifically, IGF-I increases FSH-induced estradiol production by bovine granulosa cells [39], increases LH-induced androstenedione production and LH receptors in bovine thecal cells [40], and increases proliferation of bovine granulosa [41] and thecal cells [40] in vitro. Consistent with the hypothesis that reduced plasma IGF-I may alter ovarian function, increases in plasma IGF-I concentrations paralleled increases in the maximum diameter of the dominant follicles during realimentation of HGAIN and LGAIN. In addition to direct

ovarian actions, IGF-I may affect pituitary [42, 43] and hypothalamic [44–46] function.

The magnitude of the preovulatory increase in estradiol was less in HGAIN and LGAIN compared with M heifers. Reduced concentrations of estradiol preceding the first ovulation compared with subsequent ovulations have been observed in postpartum beef cows [9, 27]. In addition, intrafollicular concentrations of estradiol are less in preovulatory follicles of postpartum beef cattle destined to form short lifespan CL compared to those destined to form normal lifespan CL [47–49]. Reduced secretion of estradiol by preovulatory follicles of realimentated heifers compared with maintenance heifers in the present study was associated with short cycles in 9 of 11 heifers. The other 2 heifers developed a luteinized follicle. Short luteal phases have been observed in cattle during puberty (for review see [50]) and during resumption of cyclicity after realimentation of nutritionally anestrous beef heifers [10].

Concentrations of FSH in serum of nutritionally induced anovulatory heifers and preceding resumption of ovulation after realimentation were similar when compared with those of cycling heifers fed maintenance diets at the same stage of follicular growth. This is in agreement with a preliminary study indicating that concentrations of FSH are similar during normal estrous cycles, nutritionally induced anestrus, and resumption of cyclicity after realimentation of nutritionally anestrous heifers [51].

During the estrous cycle before these nutritionally restricted heifers became anovulatory, concentrations of glucose and insulin in plasma were 78% and 50%, respectively, of the concentrations in M heifers [11]. Realimentation of nutritionally induced anestrous heifers resulted in increased insulin and glucose concentrations in plasma with no change in insulin and glucose between W-2 and W0. Realimentation of cattle that had been chronically underfed [52, 53] increased concentrations of insulin and glucose to concentrations similar to those in control steers within 30 days. This indicates that insulin and glucose concentrations may not be key factors that time the resumption of ovulation after realimentation of nutritionally induced anovulatory beef heifers. Insulin and glucose concentrations in the postpartum beef cows are not predictive of luteal activity [54], and intracerebraventricular infusion of insulin in growth-restricted ovariectomized ewes did not alter LH secretion [55].

Realimentation of nutritionally induced anestrous heifers resulted in decreased concentrations of NEFA in plasma; however, a gradual increase in NEFA concentrations occurred preceding resumption of ovulation. Plasma concentrations of NEFA are inversely related to feed intake or energy balance in ruminants [28, 56]. Feed restriction reduces resting metabolic rate in heifers [53] and steers [57]. Although LH pulse frequency was negatively correlated with plasma concentrations of NEFA in postpartum beef cows [26], it is unlikely that changes in systemic NEFA concentrations have a direct role in the regulation of ovarian function, since infusion of NEFA in ovariectomized lambs did not alter pulsatile secretion of LH [58].

In summary, maximum size, growth rate, regression rate, and persistence of dominant follicles increased gradually during realimentation of nutritionally induced anovulatory heifers. Increased concentrations of LH in serum during realimentation are associated with increased concentrations of estradiol in plasma. Concentrations of IGF-I, glucose, and insulin increased when feed intake was increased; but only concentrations of IGF-I increased gradually during successive follicular waves, as serum concentrations of LH increased, until the DF ovulated. The association between the gradual increases in plasma concentrations of IGF-I, serum LH, and size of the DF during realimentation of anovulatory heifers indicates that IGF-I may be one of many signals that influence secretion of LH and ovarian function.

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