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Original article

Follicular dynamics, plasma metabolites, hormones and insulin-like growth factor I (IGF-I) in lactating cows with positive or negative energy balance during the preovulatory period

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Summary — The effect of dietary energy balance (EB) on growth of ovarian follicles was tested. Cows (n = 9) were fed a high energy diet (HE diet; positive EB; n = 4) or switched to a low energy diet (LE diet; negative EB; n = 5) during the preovulatory period. Non-esterified fatty acids (NEFA) were greater in cows fed the LE diet. Concentrations of luteinizing hormone (LH) were similar in HE and LE cows. However, the growth of preovulatory follicles in cows fed the LE diet was 50% that of cows fed the HE diet. Insulin-like growth factor-I (IGF-I) in plasma was less in LE-fed cows compared with HE-fed cows, and plasma IGF-I was positively correlated to estrogen: progesterone ratio in follicular fluid of dominant follicles. In summary, slower follicular growth in cows fed an LE diet occurred despite normal plasma LH and coincided with reduced IGF-I and elevated NEFA in plasma.

energy balance / ovarian follicle / IGF-I / lactation / bovine

Résumé — Dynamique folliculaire, métabolites dans le plasma sanguin, LH et IGF-1 chez les vaches en lactation recevant un régime à haute ou à basse valeur énergétique pendant la période préovulatoire. *L'effet de l'apport énergétique alimentaire (EA) sur la croissance des follicules ovariens a été étudié. Des vaches Holstein (n = 9) ont reçu un régime à haute valeur énergétique (régime HE; excédent EA; n = 4). Cinq d'entre elles sont passées à un régime à basse valeur énergétique (régime BE; deficient EA) pendant 4 jours avant l'ovulation. Les acides gras non estérifiés (AGNE) étaient plus élevés chez les vaches nourries avec le régime BE. La concentration de l'hormone luteinisante (LH) est restée la même dans les 2 lots de vaches. Cependant, la croissance des follicules préovulatoires chez les vaches nourries avec le régime BE a été réduite de 50% par rapport aux vaches nourries avec le régime HE. Le facteur de croissance IGF-1 a baissé dans le plasma des vaches soumises au régime BE mais pas dans celui des vaches soumises au régime HE. La concentration d'IGF-1 plasmatique est corrélée positivement avec le rapport d'œstrogène/ progestérone dans le liquide folliculaire des follicules dominants. En résumé, la croissance folliculaire est plus lente chez les vaches qui reçoivent le régime BE malgré une secrétion normale de LH; elle coïncide avec une réduction d'IGF-1 et une augmentation des AGNE dans le plasma sanguin.*

apport énergétique alimentaire / follicule ovarien / IGF-1 / lactation / bovin

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INTRODUCTION

The initiation of ovarian follicular growth during the early postpartum period of dairy cows may be directly affected by numerous hormones or metabolites whose secretion depends on the extent of negative energy balance experienced by the individual cow. These would include luteinizing hormone (LH; Stevenson and Britt, 1979; Nett, 1987; Butler and Smith, 1989) as well as growth factors (insulin (Poretsky and Kalin, 1987) or IGF-I (Gluckman *et al,* 1987; Hammond *et al,* 1988)) and energy metabolites (nonesterified fatty acids [NEFA] or glucose). Plasma concentrations of IGF-I and insulin are low during periods of negative energy balance (Gluckman *et al,* 1987) and since these growth factors are critical to the development of the follicle (Adashi *et al,* 1985; Hammond *et al,* 1988), their low concentration in the plasma may affect postpartum ovarian recrudescence. Although direct effects of low plasma IGF-I or insulin concentration on the ovary are not known, IGF-I is higher in the blood and follicular fluid of cattle selected for enhanced follicular growth and development (*ie,* multiple ovulation; Echternkamp *et al,* 1990). Therefore, the ovary seems to be responsive to changes in concentrations of growth factors in the blood. The objectives of this study were to examine changes in hormones and growth factors in cows undergoing rapid changes in nutrient partitioning and to relate these to programmed changes in spontaneous preovulatory follicular development observed in the ovary. This information may elucidate additional factors controlling growth and development of follicles in postpartum cows in negative energy balance.

MATERIALS AND METHODS

Animals

Ten lactating Holstein cows (approximately 150 d of lactation) at the University of Florida, Dairy Research Unit (Hague, FL, USA) were used. All cows had a corpus luteum at the start of the experiment. Cows were milked and fed twice daily. Feed consumption was monitored using self-activated feeding stations (American Calan Inc, Northwood, NH, USA). Cows were trained to feeding stations during the week prior to the initiation of dietary treatments. Daily energy balance (difference between dietary energy consumed and the amount of energy utilized for maintenance and milk production) was calculated from milk production and composition, individual feed energy consumption, feed energy content, and body weight using formulas described previously (Lucy *et al,* 1991a).

Experimental design

Animals were injected with 8 μg Buserelin (Receptal, Hoechst-Roussel Agri-Vet Co, Somerville, NJ, USA) and a controlled internal drug release device (CIDR, 1.9 g progesterone, Carter-Holt Plastics Molding Co, New Zealand) was inserted into the vagina. Seven days later, animals were injected with 25 mg of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Lutalyse, UpJohn Co, Kalamazoo, MI, USA) and the CIDR was removed 48 h later. This treatment sequence was designed to synchronize the growth of the preovulatory follicle. Buserelin (GnRH agonist) releases luteinizing hormone from the pituitary and causes the luteinization of large dominant follicles. Prostaglandin $F_{2\alpha}$ was injected to cause luteolysis of Buserelin-induced corpora lutea or corpora lutea present at the start of the experiment. Removal of the CIDR two days after $PGF_{2\alpha}$ was designed to synchronize the expression of estrus and ovulation. This treatment sequence was initiated at random times with respect to previous estrus, and similar regimens have been shown to synchronize estrus in a large group of randomly cy-

cling heifers (Thatcher *et al,* 1989). Ovaries were removed by flank incision under local anesthesia, 36 h after CIDR removal but prior to the expression of estrus.

Cows were chronically fed a diet which was balanced to meet nutritional requirements of lactation and continued for the first 5 days of the follicular synchronization. On day 6, five cows were fed the previous diet (high energy, HE diet; 1.72 Mcal/kg DM) while 5 cows were switched to a low energy diet consisting of corn silage and minerals (LE diet; 6.4 kg DM offered daily; 1.50 Mcal/kg DM; table I). Cows in each treatment group were similar in body weight at the start of the trial (mean = 568 and 544 kg for LE and HE-fed cows, respectively). This amount of energy intake was designed to induce the energy deficit experienced by cows in early lactation. Cows were fed these diets for 4 d (until the time of ovariectomy). Ten ml of blood were collected daily by coccygeal venipuncture into heparinized tubes (Vacutainer, Becton Dickinson, East Rutherford, NJ, USA; 143 U heparin per 10 ml sample) and plasma harvested by centrifugation (3 000 *g* for 30 min). On the first day of the dietary change, as well as the day of ovariectomy, each cow was fitted with a jugular cannula and 10 ml of blood were collected once every 10 min for 8 h for analysis of luteinizing hormone (LH) concentrations in plasma.

Ovaries were examined by ultrasonography using an Equisonics LS 300A linear array scanner equipped with a 7.5 Mhz transducer (Tokyo Kieki, Tokyo, Japan) in mid-morning on each day from the time of Buserelin injection and CIDR insertion until ovariectomy. Size and number of ovarian follicles > 3 mm were recorded on detailed follicular maps designed to identify specific large follicles (> 5 mm) on repeated days. In this way, the size of the largest and second largest follicles could be followed during the preovulatory period. Large preovulatory follicles identified during ultrasonography were relocated visually (based on follicular maps) at ovariectomy and follicular fluid aspirated.

Table I. Composition of experimental diets expressed as percentage of dry matter.

Ingredient	*Diet*	
	High energy	*Low energy*
Corn silage	27.9	96.6
Corn	20.5	0.0
Whole cottonseed	12.2	0.0
Distillers grains	15.4	0.0
Soybean meal	8.3	0.0
Alfalfa hay	12.2	0.0
Minerals/vitamins	3.1	0.0
Dicalcium phosphate	0.2	1.7
TM salt	0.3	1.7

Analysis of blood hormones and metabolites

Concentrations of plasma glucose were measured in daily samples using the Sigma Chemical Co (St Louis, MO, USA) kit No 510 (glucose oxidase/peroxidase colorometric method). Plasma concentrations of NEFA were measured using a modified procedure of the NEFA C kit (Wako Pure Chemical Industries, Ltd, Osaka, Japan) which allowed for analysis of small volume samples. Plasma concentrations of triglyceride were measured by the method described by Foster and Dunn (1973). Plasma concentrations of insulin (Collier *et al,* 1982) and growth hormone (GH; Badinga *et al,* 1991) were determined by radioimmunoassay. All samples were measured in 1 assay and the intra-assay coefficient of variation was 12.4 and 9.7% for insulin and GH assays, respectively. Plasma concentrations of IGF-I were determined by radioimmunoassay as described by Lee *et al* (1990). The intra- and inter-assay coefficients of variation were 4.2 and 7.5%, respectively. Plasma concentrations of progesterone were measured in a single assay using procedures described previously (Knickerbocker *et al,* 1986). Intra-assay coefficient of variation was 6%. Concentrations of estradiol in plasma were determined by a single antibody radioimmunoassay (Badinga *et al,* 1992). All samples were analyzed in 1 assay. The sensitivity and intra-assay coefficient of variation were 0.5 pg/ml and 6.0%, respectively. Plasma LH was measured by radioimmunoassay as described by Lucy *et al* (1992). Intra- and inter-assay coefficients of variation were 9.6 and 8.3%, respectively. The concentrations of LH across the blood collection period were subjected to a peak identification algorithm (Pulsar program; Meriam

and Wachter, 1982) for the determination of mean concentration, smooth mean concentration, number of episodic events (hormone peaks), peak amplitude, and peak length. Due to the absence of pulsatility in LH at both sampling periods, 1 cow was discarded from the HE control group. Thus, all experimental responses were obtained from 4 (HE) and 5 (LE) cows, respectively.

Statistical analysis

Data were analyzed using the General Linear Models Procedure of SAS (1987). Hormone, metabolite, and follicular responses were analyzed as a split plot with repeated measures over time. The mathematical model included effects of diet, cow-within-diet, day, diet-by-day interaction, and residual. Significance of the main effect of diet was tested using cow-within-diet as the error term, while other terms were tested with the residual. Unless stated otherwise, significance was declared at $P < 0.05$. The number of follicles within each size class (class 1: 3 to 5 mm; class 2: 6 to 9 mm; class 3: 10 to 15 mm; class 4: > 15 mm) after treatment with Buserelin was analyzed using a model which included the effects of diet, cow-within-diet, day, follicular size class, and interactions of these main effects. Data was analyzed from Buserelin injection to ovariectomy as well as only during the dietary treatment period. The growth of the preovulatory follicles as well as the decline in size of the second largest follicle was analyzed by tests of homogeneity of regression (Wilcox *et al*, 1990). Essentially, a single line was fitted to the pooled data (HE and LE cows) and then the gain (reduction in error variance) for fitting individual curves (HE and LE cows, separately) was tested.

RESULTS

Follicular populations

There was a day-by-class interaction ($P < 0.05$) for numbers of follicles after injection of Buserelin (fig 1). Two days after injection of Buserelin, numbers of class 3

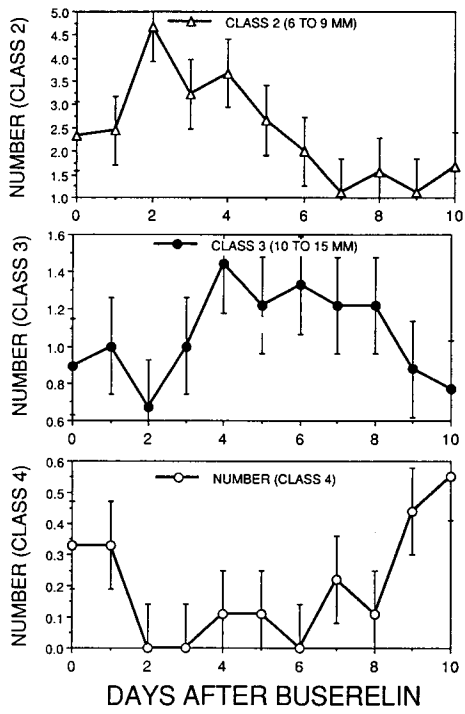

Fig 1. Average number of class 2 (6 to 9 mm), class 3 (10 to 15 mm), and class 4 (> 15 mm) follicles per cow after the initiation of follicle synchronization on day 0 (GnRH agonist injection (8 μg Buserelin)). Error bars represent standard error of the least square mean.

(10–15 mm) and 4 (> 15 mm) follicles decreased to a minima of 0.7 ± 0.3 and 0 ± 0.1 follicle per cow. The decline in the number of large follicles was followed by an increase in the number of class 2 follicles (6–9 mm) to a maxima of 4.7 ± 0.7 follicles per cow after 2 d. Mean number of class 2 follicles then declined to a minimum of 1.1 ± 0.7 after 7 d. Numbers of class 3 follicles per cow subsequently increased and then declined. Number of large follicles per cow subsequently increased and then declined. Number of

large follicles per cow (class 4) increased to maximum of 0.6 ± 0.1 follicles per cow at the end of the synchronization period.

Average number of follicles per cow (> 3 mm) increased during the dietary period in cows fed the LE diet from 5.2 ± 0.8 on d 0 to 7.4 ± 0.8 on d 4. At the same time, the average number of follicles per cow tended to decrease in cows fed the HE diet (diet-by-d, $P = 0.10$) from 7.6 ± 0.8 on d 0 to 4.0 ± 0.8 on d 4. Diet-by-class or diet-by-d-by-class interactions were not detected ($P > 0.10$) in the analysis of follicular populations during the dietary period.

Energy balance, plasma metabolites and hormones

Milk production averaged 16.7 ± 1.1 kg/d for HE-fed cows and 10.1 ± 1.0 kg/d for LE-fed cows. Calculated energy balance averaged –7.0 ± 1.1 Mcal/d in cows fed the LE diet and +5.2 ± 1.2 Mcal/day in cows fed the HE diet ($P < 0.001$). During the dietary period, plasma NEFA were higher in cows fed the LE diet than for cows fed the HE diet (547 ± 94 *vs* 326 ± 102 μEq/l; diet-by-d, $P < 0.05$). Mean concentration of insulin in plasma was similar for cows fed different diets and averaged 1.78 ± 0.12 ng/ml. Across diets, concentrations of insulin in plasma tended to increase ($P < 0.10$) during the preovulatory period (1.4 ± 0.3 ng/ml (d 2) to 2.4 ± 0.3 ng/ml (d 4)). Plasma triglycerides were similar in cows fed the LE (15 ± 3 mg%) and HE (21 ± 3 mg) diets. Plasma glucose was not affected by dietary treatment and average 73.4 ± 5.1 and 72.1 ± 4.7 mg% for cows on HE and LE diets, respectively.

Mean LH concentration (0.7 ± 0.2 *vs* 1.4 ± 0.2 ng/ml; $P < 0.07$), smoothed mean LH concentration (0.5 ± 0.1 *vs* 0.9 ± 0.1 ng/ml; $P < 0.07$) and number of LH peaks per hour (0.4 ± 0.1 *vs* 0.8 ± 0.1; $P < 0.05$) increased from dietary treatment d 0 to d 4 (table II). Cows fed the HE ($n = 4$) and LE ($n = 5$) diets were similar in terms of mean LH concentration, smoothed mean LH concentration, number of LH peaks, peak amplitude, and peak length. However, residual variance for LH concentrations on d 4 was greater ($\sigma_{LE}^2 / \sigma_{HE}^2 = 5.45$, $P < 0.001$) for LE cows as 2 LE-fed cows (8981 and 8986) did not have regular pulsatile LH secretion on d 4. Low energy-fed cow 8981 had pulsatile concentrations of LH in plasma during

Table II. Characteristics of luteinizing hormone secretion (determined by Pulsar Algorithm) after 0 and 4 days of dietary feeding for cows on the low and high energy diets.

Diet	*Day*	*Data mean (ng/ml)*		*Smoothed mean (ng/ml)*		*No of peaks (per h)*		*Amplitude (ng/ml)*		*Peak length (min)*	
		Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
LE	0	0.7	0.3	0.4	0.2	0.4	0.1	1.3	0.4	48	11
HE	0	0.7	0.3	0.5	0.2	0.4	0.1	1.4	0.4	52	13
LE	4	1.3	0.3	0.8	0.2	0.7	0.1	2.0	0.5	29	11
HE	4	1.4	0.4	0.9	0.2	0.8	0.1	1.6	0.6	44	13

LE, $n = 5$; HE, $n = 4$.

the first window bleed (d 0) but LH pulses ended 5 h into the second window bleed (d 4). In addition, LE-fed cow 8986 had a normal frequency of LH pulses during the first window bleed but only a single LH pulse during the second window bleed (d 4).

Concentrations of plasma IGF-I tended ($P < 0.08$) to be lower for LE-fed cows compared with HE-fed cows while plasma GH was not different in cows on HE or LE diets (table III). A significant negative correlation (tested by linear regression analysis) between IGF-I and GH in plasma was detected ($Y = 84.3 - 7.0X$; $P < 0.001$; $R^2 = 0.46$; Y = IGF-I ng/ml; X = GH ng/ml). Plasma progesterone concentrations increased ($P < 0.001$) following injection of Buserelin and CIDR insertion to a maximum of 7.6 ± 0.5 ng/ml after 2 d. Following injection of $PGF_{2\alpha}$, mean plasma progesterone declined ($P < 0.001$) from 5.7 ± 0.6 ng/ml (d 1 of diet) to 2.4 ± 0.6 ng/ml (d 2). A further decline in progesterone occurred after removal of the CIDR on d 3 (1.3 ± 0.6 ng/ml) to a concentration of 0.7 ± 0.6 ng/ml on d 4. Concentrations of progesterone in plasma were similar ($P > 0.10$) for LE and HE-fed cows before (4.6 ± 1.2 and 7.3 ± 1.3 ng/ml, respectively) and after (2.8 ± 0.7 and 3.6 ± 0.8 ng/ml, respectively; table III) initiation of dietary treatments. Plasma estradiol increased with day of diet (day linear, $P < 0.01$), but was similar for cows fed the HE or LE diets (table III). Residual variance for mean estradiol during the dietary period was greatest ($P < 0.01$) for cows fed the LE diet.

Growth of largest and second largest follicles

Diameter of the largest follicle at the start of dietary treatment was similar ($P = 0.43$) for LE-fed and HE-fed cows (mean = 8.25 ± 2.0 mm and 10.6 ± 1.9 mm, respectively). During the dietary period, the largest follicles increased in size ($P < 0.001$), but growth of dominant follicles was slower ($P < 0.01$; table IV) in cows fed the LE diet (0.9 mm/day) compared with the HE diet

Table III. Concentration of plasma insulin-like growth factor I (IGF-I; ng/ml), growth hormone (GH; ng/ml), progesterone (ng/ml) and estradiol (pg/ml) in cows fed the low (LE) and high (HE) energy diets starting on dietary day 0.

Hormone	*Dietary day*						
	Diet	*0*	*1*	*2*	*3*	*4*	*SEM*[a]
IGF-I	LE	64.7	53.9	47.8	50.0	42.0	6.4
IGF-I	HE	65.3	69.3	69.2	66.4	71.4	6.4
GH	LE	3.5	2.7	3.5	3.5	4.2	1.4
GH	HE	6.7	2.9	3.0	2.9	4.1	1.4
Progesterone	LE	4.6	4.6	2.3	1.3	1.0	0.8
Progesterone	HE	6.9	7.1	2.4	1.2	0.4	0.9
Estradiol	LE	2.6	2.8	4.9	6.8	5.6	1.9
Estradiol	HE	1.8	4.1	5.5	5.9	6.3	1.7

[a] Pooled SEM for diet-by-day interaction.

Table IV. Analysis of variance for homogenity of regression for dominant and second largest follicles during the dietary treatment (TRT) period.

Dependent variable	*Regression*	*Curve*	*Residual*				
			df	*ss*	*ms*	F *ratio*	P
Largest follicle	Linear	Pooled	35	72.1	2.1		
		TRT	34	56.8	1.7		
			1	15.3	15.3	15.3/1.7	0.01
Second largest follicle	Linear	Pooled	35	94.7	2.7		
		TRT	34	85.5	2.5		
			1	9.2	2.7	9.2/2.5	0.10

(1.8 mm/day; fig 2) and a diet-by-day interaction was detected ($P < 0.05$). In addition, the second largest follicles decreased in size during the dietary period ($P < 0.05$) and the decrease in size tended to occur at a faster rate (–0.9 *vs* 0.3 mm/day; table IV; $P < 0.10$) in cows fed the HE diet compared with the LE diet (fig 2). The relationship in size between the largest follicle and the second largest follicle differed between the HE and LE diets when examined by a covariance analysis for the dietary periods ($P < 0.01$). In the HE diet, a 1.4 mm increase in size of the largest follicle was associated with a 1.0 mm decrease in the size of the second largest follicle (–1.44; $P < 0.01$); for the LE diet there was no significant association between the size of the largest and second largest follicle (–0.08; $P < 0.70$).

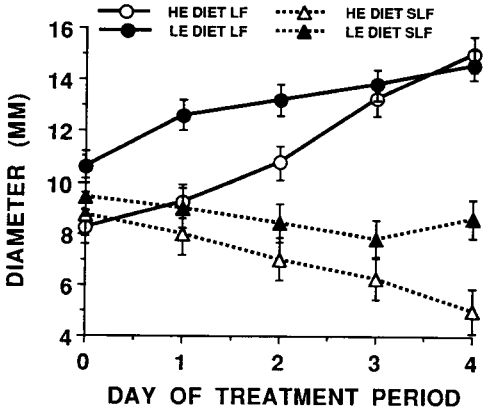

Fig 2. Growth (mm) of the preovulatory follicle and decline in size of the second largest follicle in cows fed the high ($n = 4$, anestrous cow removed) and low ($n = 5$) energy diets. Error bars represent standard error of the least square mean.

IGF-I concentrations in follicular fluid on the day of ovariectomy are shown in table V. The results of 6 cows are presented only because the largest follicles in 3 cows (1 520, HE diet; 956 and 1 482, LE diet) were ruptured at the time of ovariectomy. Five of the six largest follicles collected had estradiol to progesterone ratios in follicular fluid greater than 1.0 (3 of 3 HE-fed cows and 2 of 3 LE-fed cows). The IGF-I concentration in follicular fluid ranged from 18.7 ng/ml to 131.6 ng/ml. There tended to be a positive correlation ($P < 0.06$, analyzed by linear regression)

Table V. Insulin-like growth factor - I (IGF-I, ng/ml) in plasma and follicular fluid of largest follicles and estradiol to progesterone ratio for follicular fluid of largest follicles in cows on the day of ovariectomy (d 4 of dietary feeding).

Cow	*Diet* [a]	*Size* [b]	*E:P* [c]	*IGF-I*	
				Plasma	*FF*
1443	LE	16	17.2	76.7	55.8
8981	LE	21	0.1	50.4	131.6
8986	LE	15	1.4	16.9	18.7
1376	HE	19	3.1	61.0	53.9
1466	HE	12	4.1	83.7	99.9
1498	HE	14	20.6	140.9	69.7

[a] LE = low energy diet, HE = high energy diet; [b] follicular diameter (size); [c] ratio of estradiol to progesterone in follicular fluid.

between estrogen:progesterone ratio in follicular fluid and plasma IGF-I ($Y = -4.44 \pm 0.17X$; $P < 0.06$; $R^2 = 0.64$; Y = E:P ratio; X = IGF-I in plasma). There was no correlation ($P > 0.10$) between IGF-I in plasma and follicular fluid estrogen to progesterone ratio and IGF-I in follicular fluid, or diameter of the follicle and IGF-I in serum or follicular fluid.

DISCUSSION

Growth rates of potentially ovulatory follicles were less in cows fed the LE diet compared with cows fed the HE diet. This suggests that acute growth of preovulatory follicles can be affected by short-term changes in energy balance. Lamond (1970) previously reported that short-term energy restriction decreased ovulation rate in heifers treated with pregnant mare's serum gonadotropin. It is not clear what metabolic or hormonal factors cause this slower terminal growth of large preovulatory follicles. Concentrations of LH in plasma were unchanged when measured by radioimmunoassay. Changes in the bioactivity of gonadotropins (not measured in this trial) may have affected follicular growth. Plasma IGF-I was lower in LE-fed cows and there was a positive correlation between estrogen:progesterone ratio in follicular fluid and plasma IGF-I. Plasma NEFA were higher in LE-fed cows, suggesting that fat was mobilized in response to diet-induced negative energy balance. However, plasma glucose and insulin concentrations were not changed by dietary treatments. Collectively, the results suggest a tentative relationship between nutrition, concentration of plasma IGF-I, plasma metabolites (*eg* NEFA), and growth of both dominant and subordinate follicles. Perhaps reduced nutrient intake alters IGF-I in blood (described by Gluckman *et al,* 1987; caused by reduced concentration of GH receptors in the liver) to markedly reduce follicular growth. A clear evaluation of whether decreased nutrient intake altered concentration of IGF-I in follicular fluid was not evident in the present study. Similar experiments, with a greater number of animals, need to be performed to evaluate responses within the follicle. Spicer *et al* (1991) reported that short-term fasting (48 h) in heifers decreased plasma IGF-1 but did not alter IGF-I concentrations in follicular fluid.

Size of the largest follicle appeared to converge to a common size in this trial. One possibility, suggested by these data, is that the largest follicles in this trial mature to a similar size (15 mm) because of an earlier initiation of follicular waves in LE-fed cows. However, when lactating cows from the same herd were similarly treated with CIDRs and $PGF_{2\alpha}$ during the preovulatory period, the largest follicles reached a maximum size of 18 mm (Lucy *et al,* 1991a, b) and 19.3 mm (Lucy *et al,* 1990). Furthermore, visual inspection of

data from individual cows showed that a high proportion of dominant follicles in cows fed the LE diet did not grow during the preovulatory period (4 out of 5 cows). Therefore, the alternative interpretation (described above) is that growth of the dominant follicle was slowed in LE-fed cows which led to the convergence of mean diameter across the 2 groups. An additional 1 or 2 days of dietary treatment may have increased the sensitivity of the design by allowing further maturation of dominant follicles.

The second largest follicle decreased in size at a greater rate in cows fed the HE diet. In addition, the total number of follicles per cow increased across days of the preovulatory period in LE-fed cows while decreasing across days in cows fed the HE diet. The decrease in size of the second largest follicle during a follicular wave and a reduction in the total number of follicles on the ovary were associated with the development of physiologically active dominant follicles (Lucy *et al,* 1990). Therefore, when compared to LE-fed cows, cows fed the HE diet developed dominant follicles which more effectively controlled the growth of other follicles on the ovary and may have been more physiologically active. The diameter of the second largest follicles increased (albeit nonsignificantly) on d 5 in LE-fed cows. This was unexpected, and when these data are removed from the analyses, the decline in the size of the second largest follicle is similar between HE and LE-fed cows. Therefore, while the second largest follicle data should be interpreted cautiously, other data (*ie*: changes in total numbers of follicles, and the negative relationship between the largest and second largest follicle in the HE diet but not the LE diet) support the concept that a more physiologically active follicle developed in HE-fed cows.

Changes in the dynamics of follicular populations within the ovaries of cows in this trial (fig 1) were consistent with known effects of a GnRH agonist on the ovary (Thatcher *et al,* 1989). Following an injection of Buserelin, there was a rapid decline in the number of large follicles (> 10 mm) which were either luteinized or ovulated (table II). This was followed by an increase in the number of class 2 follicles on the ovary (6–9 mm). This increase was probably stimulated by the functional loss of large follicles (luteinizing by Buserelin-induced LH release) thus releasing these smaller follicles from the effects of follicular dominance (Ireland and Roche, 1987). Alternatively, small follicles may have been stimulated by GnRH injection, independent of the effects of follicular dominance. Eventually, smaller follicles grew into the larger class 3 dominant follicles (10–15 mm) resulting in a decline in the number of class 2 follicles per cow. Finally, as cows entered the preovulatory period the number of class 4 follicles (> 15 mm) increased. Thus, due to the experimental programming of follicular growth, either a class 3 or 4 follicle was present as the time of ovariectomy approached.

In conclusion, short-term feeding of a diet low in energy caused the preovulatory follicle to grow at a slower rate and the second largest follicle to decrease in size at a slower rate when compared to cows fed a diet high in energy. Cows on the LE diet had a greater concentration of NEFA, and decreased concentrations of IGF-1 in plasma. Changes in follicular growth induced by diet were not associated with glucose or insulin. These results infer that plasma IGF-I may modulate growth and development of follicles in cows experiencing a negative energy balance.

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