Ovarian Follicular Development in Prepubertal Heifers Is Influenced by Level of Dietary Energy Intake

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Ovarian Follicular Development in Prepubertal Heifers Is Influenced by Level of Dietary Energy Intake[1]

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ABSTRACT

Objectives were twofold: 1) to determine the chronology of development of dominant ovarian follicles during the peripubertal period in heifers and 2) to determine whether feeding a diet with low energy content that delays onset of puberty alters chronology of dominant ovarian follicular development in peripubertal heifers. Ten heifers of composite breeding (1/4 Angus, 1/4 Hereford, 1/4 Red Poll, 1/4 Pinzgauer) were randomly assigned, at 8 mo of age, to receive a diet designed to produce 0.9 (n = 5) or 0.3 (n = 5) kg body weight gain per day for the duration of the experiment. To characterize changes in size of ovarian follicles, real-time linear ultrasonography of ovaries was conducted in all heifers every other day until puberty occurred. Blood samples were collected weekly to determine concentrations of progesterone and 17β-estradiol. Determination of time of puberty was based on increased concentrations of progesterone, ultrasound depiction of ovulation, and subsequent presence of a corpus luteum. Size of the dominant ovarian follicles differed prior to puberty ($p < 0.03$); diameter of the dominant ovarian follicle was greater in all heifers as the first ovulation approached as compared to earlier in prepuberty. Heifers fed the greater amount of energy exhibited larger dominant ovarian follicles at a younger age in comparison to heifers fed the lower amount of energy. Age and weight at puberty differed among heifers receiving diets with greater energy content compared to those receiving diets with lower energy content (372 ± 7 and 435 ± 9 days of age at puberty, respectively, $p < 0.003$; 263 ± 7 vs. 221 ± 3 kg, respectively, $p < 0.01$). Persistence of dominant ovarian follicles did not differ among heifers fed the diet with greater energy compared to heifers fed the lower-energy diet. Neither growth rate of dominant ovarian follicles nor rate of regression differed consistently between treatment groups. Additionally, size of the ovulatory follicle at puberty did not differ between treatment groups; however, 4 of the 5 heifers fed the diet with greater energy content had a luteal phase of abnormal length subsequent to the first ovulation, whereas all of the heifers fed the low-energy diet had luteal phases of normal length subsequent to first ovulation. We conclude that the chronology of development of dominant ovarian follicles differs in heifers fed diets of different energy content, with maturational events being delayed in heifers fed a lower-energy diet.

INTRODUCTION

Recently it has been determined that ovarian follicles in prepubertal heifers grow and regress in waves, following the same process as in adult animals [1–5]. However, in adult animals, these follicles develop to the ovulatory stage. Heifers as young as two weeks of age exhibit wave-like patterns of ovarian follicular growth [3]. Possible variations in characteristics of these waves as puberty approaches still remain to be defined. Results from our laboratory have shown that ovaries of prepubertal heifers contain at least one follicle greater than 7 mm in surface diameter, by 100 days prior to the first ovulation [6].

There is an increase in secretion of LH as puberty approaches, particularly during the last 50 days prior to the pubertal ovulation [7–10]. These increases in LH can be delayed when heifers are subjected to restrictions in dietary energy intake [8–10]. On the basis of these results and of our current knowledge of the role of LH in regulating growth of ovarian follicles, we predicted that waves of follicular growth during pubertal development would differ, in relation to the patterns of LH to which the ovaries are exposed, when heifers were fed high as compared to low amounts of energy during sexual maturation. We hypothesized that dominant ovarian follicles would have a larger diameter as heifers approached puberty. We also hypothesized that lower levels of dietary energy would alter the chronological pattern of ovarian follicular development. Therefore, the objective of the present experiment was to determine changes in development of dominant ovarian follicles and to ascertain whether feeding a diet with low energy content that delayed onset of puberty would alter chronology of development of dominant follicles in peripubertal heifers.

MATERIALS AND METHODS

Ten prepubertal 8-mo-old heifers of composite breeding (1/4 Angus, 1/4 Hereford, 1/4 Red Poll, and 1/4 Pinzgauer) were randomly assigned to receive a diet designed to produce 0.9 kg/day (n = 5; 67% cracked corn, 23% corn cobs, 8% soybean meal, 1% calcium diphosphate, and 1% sodium chloride) or 0.3 kg/day (n = 5; 18% cracked corn, 62% corn cobs, 9% soybean meal, 9% molasses, 1% calcium diphosphate, and 1% sodium chloride) of body weight gain for the duration of the experiment. Diets were balanced equally for all components other than energy. Heif-

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ers were individually fed daily and weighed weekly to ensure that the target rates of gain were maintained.

To characterize changes in size of ovarian follicles, real-time linear ultrasonography was conducted in all heifers every other day until puberty was attained. A heifer was considered to have reached puberty when concentrations of progesterone rose above 1 ng/ml in two consecutive samples after detection of an ovulated follicle by ultrasonography. Observations were terminated for individual heifers when both concentrations of progesterone and ultrasound depiction of two consecutive ovulations, as determined by the presence of a corpus luteum, indicated that onset of estrous cycles had occurred. The second ovulation had to take place within 25 days of the initial ovulation and subsequent development of a corpus luteum before collection of ultrasonographic data was terminated. Real-time ultrasonography was conducted with use of an Equisonic LS300 Real-Time Linear Scanner, equipped with a 7.5-MHz intrarectal transducer (Tokyo Keiki LS-300A; Products Group International, Boulder, CO).

Ultrasonography has been shown to be reliable in measuring ovarian follicles as small as 3 mm in diameter and for following changes in growth of individual follicles [11, 12]. The location of each follicle was determined using the craniocaudal and medio-lateral planes by placing the transducer over an ovary and rotating it around the long axis [12]. Although follicles were identified and measured in real time, two pictures from the sonograph of each ovary were taken with a Sony High Contrast printer (Products Group International) for further analyses. In our laboratory, these procedures have been determined to accurately predict size of dominant ovarian follicles by relating size of dissected follicles to those detected by ultrasonography [13, 14].

Development of dominant ovarian follicles was defined according to criteria of Knopf et al. [12] and Ginther et al. [15–17]. Follicular waves are characterized by the initial recruitment of a group of growing follicles from which one follicle (the dominant follicle) is selected to continue its growth while the others undergo atresia [18]. Waves of follicular development were determined by the presence of a dominant follicle; the first day that a follicle reached 5 mm in diameter marked the beginning of the wave. If the largest follicle was not detected until it reached 7 mm, then the day previous to the day of first observation of that follicle was considered as the day of initiation of the wave. A wave was considered to have ended when emergence of the dominant follicle of the following wave was detected. Each follicle greater than 5 mm was located in relation to the latero-medial, dorso-ventral, and cranio-caudal position of the ovary, as well as in relation to other structures present within the ovary (other follicles or the corpus luteum). In order to avoid the construction of preconceived patterns of follicular changes, the following criteria [12] were used: each wave was numbered and characterized according to its duration in days, the maximum size of the dominant follicle, rate of growth ([maximum size—5 mm]/number of days) and rate of decline ([maximum size—minimum size after regression has been initiated]/number of days) [15]. Ovarian follicular growth was determined individually for both the right and left ovary of each heifer.

Blood samples were collected to determine when the first luteal function occurred. Determination of onset of puberty was based on concentration of progesterone and depiction of ovulation of an ovarian follicle and subsequent development of a corpus luteum through ultrasonography. Collection of jugular blood samples occurred weekly from initiation of the experiment (January 1) until heifers achieved puberty. Samples were collected into tubes treated with a 30% solution of EDTA (50 μl/tube) and were centrifuged within 2 h of collection to minimize degradation of progesterone. Plasma was stored at −20°C until assayed.

Concentrations of progesterone [19] and 17β-estradiol [20–22] were determined by RIAs that have been previously validated in our laboratory. Intraassay coefficients of variation were 6 and 8%, and interassay coefficients of variation were 12 and 14%, respectively, for the progesterone and 17β-estradiol assays.

Data relating to ovarian follicles were averaged for individual heifers over 30-day intervals from the initiation of the study until each heifer attained puberty. Additionally, data relating to ovarian follicles were averaged for individual heifers over 30-day intervals, retrospectively, from first ovulation back in time to 120 days prior to puberty. Subsequently, data for heifers of each group were averaged for statistical analysis. Variables used to characterize waves of ovarian follicular development were subjected to analyses that compared the changes over time for heifers fed the two diets using a mixed-model analysis for repeated measures over time [23]. An analysis of variance for a completely randomized design was also utilized to determine differences due to diet; these included the fixed effects of age at puberty, rate of gain, mean concentration of progesterone, interval between follicle selection for dominance and ovulation, and length of first estrous cycle following the pubertal ovulation [24, 25]. Additionally, regression coefficients for the maximum size of the dominant ovarian follicles across time were obtained through the PROC GLM procedure of SAS.

RESULTS

Data for dynamics of ovarian follicular development for the two groups of heifers are included in Table 1. All heifers exhibited waves of follicular growth, as determined by the divergent growth of a large "dominant" ovarian follicle and numerous smaller, subordinate follicles. These large dominant follicles grew and regressed over time with a cohort of smaller follicles. Rate of regression (mm/d) did not differ among heifers in the two groups for the largest ovarian follicle. Neither growth rate (mm/d) nor duration (d)

TABLE 1. Growth rate, duration of follicular wave, maximum size of the dominant ovarian follicle, and concentration of 17β-estradiol, by month of treatment prior to puberty for heifers fed a diet of high (High) or low (Low) energy content.[a]

Month:	1	2	3	4	5	6	7
High	n = 5	n = 4	n = 3	n = 2	---[b]	---	---
Low	n = 5	n = 5	n = 5	n = 4	n = 4	n = 2	n = 2
Growth (mm/day)							
High	1.23	1.77	1.41	1.40[c]	---	---	---
Low	1.26	1.54	1.50	1.95[d]	1.70	1.72	1.30
SEM[e]	(0.13)	(0.12)	(0.13)	(0.23)	(0.14)	(0.18)	(0.26)
Duration (day)							
High	8.01	8.04	8.62	8.97	---	---	---
Low	5.97	7.43	7.66	7.39	7.43	7.96	6.68
SEM[e]	(0.68)	(0.69)	(0.79)	(1.85)	(0.76)	(0.96)	(1.36)
Maximum size (mm)							
High	11.05[c]	11.96[c]	12.14[c]	12.02	---	---	---
Low	9.16[d]	10.54[d]	10.45[d]	10.85	11.86	12.60	12.02
SEM[e]	(0.52)	(0.51)	(0.53)	(0.90)	(0.57)	(0.71)	(0.98)
17β-estradiol (pg/ml)							
High	4.13[c]	4.66[c]	3.98[c]	3.11	---	---	---
Low	2.04[d]	2.74[d]	2.83[d]	2.82	2.61	3.29	3.91
SEM[e]	(0.53)	(0.53)	(0.53)	(0.56)	(0.55)	(0.53)	(0.83)

[a]All data collected over a monthly interval were averaged for each heifer and subsequently their data were averaged to obtain values included in this table.
[b]All heifers fed the diet with greater energy content attained puberty by the fifth month of the study.
[c,d]Numbers within columns with different superscripts differ significantly ($p < 0.05$).
[e]Pooled SEM.

differed consistently over time for heifers in either treatment group. Maximum diameter of dominant follicles was smaller ($p < 0.05$) in heifers receiving the lower-energy diet than in heifers receiving the diet with greater energy when the groups were compared across months of treatment prior to onset of puberty (Table 1).

Circulating concentrations of 17β-estradiol were associated with size of the dominant ovarian follicles (Table 1). Heifers receiving the diet with greater energy content had larger-diameter dominant ovarian follicles at an earlier age, and these follicles were associated with greater circulating concentrations of 17β-estradiol. Heifers receiving the diet of lower energy content developed larger-diameter dominant ovarian follicles at a later age, and these also were associated with increasing concentrations of 17β-estradiol.

Heifers fed the two diets did not exhibit different follicular sizes when compared at fixed physiological ages (30, 60, 90, or 120 days) prior to puberty (Table 2). As heifers approached time of first ovulation (puberty), maximum size of the dominant follicle increased in a linear manner (Fig. 1). Size of dominant ovarian follicles was larger ($p < 0.03$) immediately preceding puberty as compared to earlier in the prepubertal period for both treatment groups.

TABLE 2. Growth rate, rate of atresia, duration of follicular wave, and maximum size of dominant ovarian follicle across 30-day periods of time prior to puberty for heifers fed a diet of high (High) or low (Low) energy content.[a]

Days prior to puberty	Growth (mm/day)	Atresia (mm/day)	Duration (days)	Maximum size (mm)
High (n = 5)				
≤ 30	1.71	1.65	8.42	12.18[b]
≤ 60	1.64	1.24	8.73	11.20[c]
≤ 90	1.56	1.42	9.02	10.91[c]
≤ 120	1.34	1.41	8.50	10.13[c]
Low (n = 5)				
≤ 30	1.63	1.55	9.46	12.47[b]
≤ 60	1.47	1.33	8.84	11.52[c]
≤ 90	1.41	1.46	7.63	11.25[c]
≤ 120	1.50	1.87	7.03	9.62[c]
SEM[d]	(0.38)	(0.41)	(1.62)	(1.32)

[a]All data collected over a 30-day interval were averaged for each heifer and subsequently their data were averaged to obtain values included in this table.
[b,c]Numbers within columns with different superscripts differ significantly ($p < 0.03$).
[d]Pooled SEM.

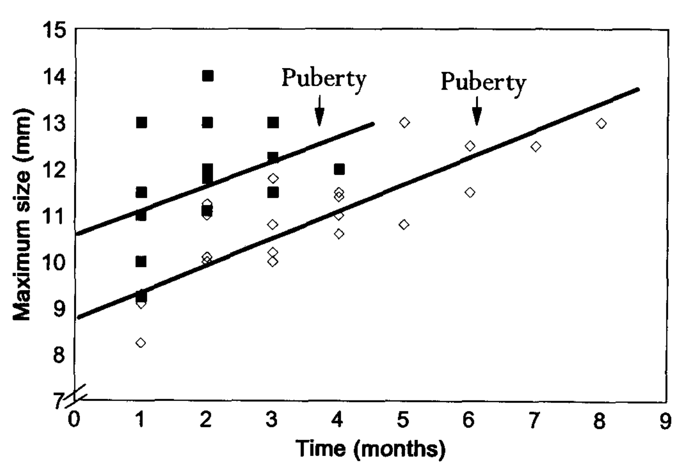

FIG. 1. Regression of size of dominant follicle across time for heifers fed a diet of either high (solid squares, $r = 0.76$) or low (open diamonds; $r = 0.43$) energy content. "Puberty" indicates mean time at which puberty occurred for each group.

TABLE 3. Beginning weight (Begin wt), final weight, total weight gained during the experimental period, gain per day, and age at puberty for individual heifers fed diets of low (Low) or high (High) energy content.

Heifer	Begin wt(kg)	Final wt(kg)	Total gain (kg)	Gain (kg/day)	Age at puberty (days)
Low					
1	177	204	27	0.30	428
2	176	218	42	0.29	399
3	165	222	57	0.32	445
4	194	226	32	0.26	403
5	156	235	79	0.34	498
Mean	174	221[a]	47[a]	0.30[c]	435[e]
SEM	(3)	(3)	(5)	(0.01)	(9)
High					
6	160	241	81	0.89	360
7	190	262	72	0.87	326
8	176	229	53	0.91	398
9	180	287	107	0.89	385
10	184	298	114	0.95	388
Mean	178	263[b]	85[b]	0.90[d]	372[e]
SEM	(3)	(7)	(6)	(0.01)	(7)

[a,b]Numbers within columns with differenct superscripts differ significantly ($p < 0.01$).
[c,d]Numbers within columns with different superscripts differ significantly ($p < 0.0004$).
[e,f]Numbers within columns with different superscripts differ significantly ($p < 0.003$).

Heifers fed the diet with greater energy content attained puberty at a younger age and heavier weight than heifers fed the diet with lower energy content (372 ± 7 vs. 435 ± 9 days of age at puberty, respectively, $p < 0.003$; 263 ± 7 vs. 221 ± 3 kg, respectively, $p < 0.01$; Table 3). Size of ovulatory follicle at puberty did not differ between groups (12.67 ± 0.89 vs. 12.40 ± 0.40 mm, respectively; Table 4). Mean interval between detection of the first ovulatory follicle and time of ovulation did not differ significantly among heifers fed the diet with greater as compared to those fed the diet of lower energy content (4.5 ± 1.5 vs. 7.0 ± 1.0 days; respectively).

Variation occurred both in length of the luteal phase of the first estrous cycle and in occurrence of estrous cycles with short luteal phases between treatment groups ($p < 0.01$; Table 5). Three of five heifers fed the diet with greater energy content had a luteal phase of shortened duration after the initial ovulation (12.0 ± 3.34 days), and one of the five heifers had an estrous cycle with a prolonged luteal phase (34 days). None of the heifers fed the diet with lower energy had luteal phases of abnormal length. Concentrations of progesterone did not differ between treatment groups, either before or after attainment of puberty. Heifers with shortened luteal phases exhibited mean concentrations of progesterone similar to those in heifers with longer luteal phases. Heifers having estrous cycles of 16–25 days in length after this first ovulation were observed to have either three or four waves of ovarian follicular growth between ovulations regardless of dietary regimen.

TABLE 4. Growth rate, duration of follicular wave, and maximum size of the first ovulatory follicle for heifers fed a diet of high (High) or low (Low) energy content.

	High	Low	SEM[a]
Growth (mm/day)	1.45	1.70	0.22
Duration (day)	7.33	5.20	1.20
Maximum size (mm)	12.67	12.40	0.54

[a]Pooled SEM.

Growth of the ovulatory follicle in millimeters per day did not differ among heifers in the two treatment groups, nor did duration in days that the ovulatory follicle remained on the ovary vary between groups. There was no difference between right and left ovaries in number of follicular waves, size of dominant follicle, or number of ovulations. It was observed, via ultrasound, that heifers in both treatment groups exhibited cloudy luteal-like anomalies on their ovaries between 1 and 3 mo prior to ovulation. These structures might have been luteinized follicles that produced small quantities of progesterone, although profiles of progesterone did not indicate changes in concentration of this hormone in these heifers during periods when these structures were detected.

DISCUSSION

Turnover of ovarian follicles was characterized by the presence of waves of follicular growth in peripubertal heifers. These waves were evident in both treatment groups and were similar in nature. Prepubertal heifers as young as 2 wk of age exhibit development of follicular waves [3]. The primary difference observed in the current study was that heifers fed the diet with low energy content had a delay in chronology of development of dominant follicles in comparison to heifers fed the diet containing greater energy. An alternate view of this would be that heifers fed the diet with the greater energy had accelerated development of maturational events associated with puberty.

On the basis of previous findings that concentrations of LH increase as puberty approaches [8, 26–28] and of recent

TABLE 5. Length of estrous cycles in days and number of waves of follicular growth for individual heifers fed diets of low (Low) or high (High) energy content.

Low			High[a]				
	Cycle 1			Cycle 1		Cycle 2	
Heifer	Length	Waves	Heifer	Length	Waves	Length	Waves
1	25	3	6	34	3	20	3
2	21	4	7	12	2	21	4
3	23	3	8	15	2	21	3
4	21	3	9	21	3	--	-
5	25	4	10	7	2	25	4

[a]Heifers exhibiting abnormal-length first estrous cycles have data for the second, normal-length estrous cycle also.

research suggesting that ovarian follicles of prepubertal heifers that develop to the point of ovulation depend on secretion of LH [29], we expected that diameter and duration of persistence of the largest dominant follicle would be greater as the first ovulation approached. Size of the dominant follicle increases gradually over time, and duration of follicular waves tends to increase over time in heifers ranging in age from 2 to 34 wk of age [3]. However, in a recent study, no increases in maximum diameter of the dominant follicle occurred in prepubertal Hereford heifers during three months prior to the pubertal ovulation [4]. We do not have an explanation for the inconsistent results of the present and previous study. In two previous studies, maximum diameter of dominant follicles was smaller in postpubertal heifers maintained on a restricted-energy diet when compared to that in heifers maintained on a diet of moderate energy content [30, 31]. Our results indicate that this was the case when the groups were compared at fixed chronological ages; however, heifers fed either diet did not exhibit different follicle sizes when compared at 30, 60, 90, or 120 days before puberty (physiological age).

Regression of the maximum size of the dominant ovarian follicle over time indicates that across time there is an associated increase in size of the dominant ovarian follicle in prepubertal heifers. No interaction between the regression coefficients was detected, suggesting that maturational events between the two groups of heifers were similar, with heifers fed the lower-energy diet exhibiting a delay in sexual maturation. Therefore, as time of puberty approached, dominant ovarian follicles of greater diameter developed in heifers of both treatment groups. This increase allowed for the greater secretion of 17β-estradiol by these follicles, which would have resulted from the decreased negative feedback of estradiol on secretion of LH prior to puberty [26–28]. The hypothalamic-pituitary axis is hypersensitive to the negative feedback of estradiol, which in turn limits secretion of LH prior to the onset of puberty [26–28]. The prepubertal decline in negative feedback of 17β-estradiol on secretion of LH is followed by a period of positive feedback postpuberty [26]. As sensitivity to 17β-estradiol lessens, there is a subsequent increase in frequency of LH pulses, particularly during the last 30 days preceding puberty. We speculate that the relatively large change in size of dominant follicles during the last 30 days prepuberty as compared to earlier prepuberty resulted from increased LH support.

The mechanism of action by which dietary intake influences ovarian follicular growth is not clear. Increases in circulating FSH have been found to precede the emergence of waves of ovarian follicular growth [32]. The peak of increase in FSH occurs between one and two days before emergence of a wave of follicular growth. Circulating concentrations of FSH begin to decrease when follicles of a wave begin to diverge into a dominant follicle and subordinate follicles [32]. However, previous studies have failed to demonstrate a significant relationship between restricted feeding and FSH concentrations in sheep [33] or cattle [34]. Insulin-like growth factor-I (IGF-I) appears to modulate effects of FSH on ovarian follicular growth and is directly influenced by plane of nutrition [35–37]. IGF-I can increase the sensitivity of granulosal cells to stimulation by FSH [35]. Speculation has occurred that dietary intake, acting through serum or intraovarian IGF-I, can influence dominance of ovarian follicles [30].

Cloudy luteal-like anomalies were observed on the ovaries of heifers from both treatment groups between 1 and 3 mo prior to the pubertal ovulation. Berardinelli et al. [38] determined that follicles can become luteinized prior to puberty and that these luteinized follicles produce progesterone. In the present study, there was no measurable increase in concentration of progesterone at the time that these anomalies were detected.

Three of the five heifers fed diets providing greater amounts of energy exhibited shortened luteal phases following their first ovulation. Shortened estrous cycles are a typical occurrence when heifers are first beginning to ovulate. Both *Bos indicus* and *Bos taurus* heifers have been reported to develop a short-lived corpus luteum at puberty, subsequent to ovulation [4, 39]. However, in the present study, none of the heifers fed the lower-energy diet were observed to have shortened estrous cycles subsequent to the pubertal ovulation. The frequency of blood sampling in this experiment may have been too infrequent to allow for short estrous cycles, less than 7 days in length, to be detected. The shortest interovulatory interval that we were able to detect via ultrasonographic depiction of ovarian events

in heifers fed the greater amount of dietary energy was 7 days in length. Increased progesterone, indicative of a functional corpus luteum, from one blood sample collected during the interovulatory interval was also detected for this heifer. The function of the short-lived corpus luteum is not yet known. Possibly it functions in establishing the ratio of estradiol to progesterone necessary to modulate frequency of LH pulses; however, this does not appear to be true in sheep [40].

Perhaps short-lived corpora lutea are not "normal," but result instead from some inadequacy; therefore they may not have a "function" per se. This would further support the argument that consumption of diets of greater energy content leads to accelerated development of maturational events, which leads to an abnormal, shortened, first estrous cycle. Maturation from both physiological and endocrinological standpoints must occur prior to puberty. A rapid increase in uterine weight occurs during the 50 days preceding the onset of puberty [41], which is associated with the increase in the maximum size of the dominant ovarian follicles and the associated increase in 17β-estradiol concentration in the present study. Uterine development during peripuberty is probably related to capacity to produce prostaglandin $F_{2\alpha}$, which is involved in luteolysis. Abnormal lengths of luteal phases subsequent to the first ovulation in heifers fed the diet with greater energy content may be related to aberrant uterine secretion of prostaglandin $F_{2\alpha}$ compared to what normally occurs during estrous cycles of adult bovine females. The heifers maintained on the lower-energy diet had a longer period of time in which they gradually overcame estradiol negative feedback. This slowing of maturational events may have allowed for suitable priming of all components of the reproductive system, and upon pubertal ovulation, these heifers exhibited estrous cycles of normal length.

In a previous study, low dietary intake reduced the diameter and persistence of follicles during the estrous cycle of postpubertal beef heifers and tended to increase the proportion of estrous cycles with three dominant follicles [30]. In the current study, no differences were detected between treatment groups as to number of follicular waves during estrous cycles of normal length. All heifers exhibiting estrous cycles between 16 and 25 days in length were observed to have either three or four waves of follicular growth between ovulations. The right ovary has been reported to be the most active ovary, with ovulation occurring from the right ovary approximately 60% of the time [42, 43]. The number of dominant follicles in the right ovary has also been reported to be greater than the number of follicles in the left ovary during the intraovulatory interval [44]. In the current study, however, larger numbers of dominant follicles did not develop in the right as compared to left ovary, nor was ovulation more likely to occur from the right ovary. This may be due, in part, to the relatively small number of animals utilized in the present study.

In summary, chronology of ovarian follicular development differed among heifers fed diets with two levels of energy content. Heifers fed greater amounts of dietary energy had larger dominant ovarian follicles at fixed points in time during the present experiment. Heifers fed the lower dietary energy developed smaller dominant ovarian follicles that were associated with a delay in attainment of puberty. Heifers in both groups developed ovulatory follicles of similar size at the pubertal ovulation, but the pubertal ovulation was an average of 63 days later in heifers fed the lower energy compared to those fed greater amounts of dietary energy.

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