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Endocrine Mechanisms of Puberty in Heifers: Estradiol Negative Feedback Regulation of Luteinizing Hormone Secretion[1]

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ABSTRACT

The hypothesis that luteinizing hormone (LH) secretion in prepubertal females is responsive to estradiol negative feedback and that decreased feedback occurs as puberty approaches was tested in heifers.

In the first experiment, seven heifers were maintained prepubertal by dietary energy restriction until 508 days of age (Day 0). All heifers were placed on a high-energy diet on Day 0 at which time they received no additional treatment (CONT), were ovariectomized (OVX) or were ovariectomized and subcutaneously implanted with estradiol-17β (OVX-E$_2$). This feeding regimen was used to synchronize reproductive state in all heifers.

A second experiment was performed with 16 prepubertal heifers using the same treatments at 266 days (Day 0) of age (CONT, OVX and OVX-E$_2$) but no dietary intake manipulation.

In both experiments, LH secretion increased rapidly following ovariectomy in OVX heifers. In the initial experiment, LH secretion was maintained at a low level in OVX-E$_2$ heifers until a synchronous rapid increase was noted coincidental with puberty in the CONT heifer. In the second experiment, LH secretion increased gradually in OVX-E$_2$ heifers and attained castrate levels coincidental with puberty in CONT heifers. A gradual increase in LH secretion occurred as puberty approached in CONT heifers.

These results indicate that: a) LH secretion in prepubertal heifers is responsive to estradiol negative feedback; and b) estradiol negative feedback decreases during the prepubertal period in beef heifers.

INTRODUCTION

A unifying concept regarding the endocrine mechanisms which control initiation of puberty in females remains obscure at present. The classical "gonadostat" theory suggests that a decrease in sensitivity of the hypothalamo-pituitary centers controlling gonadotropin secretion to estradiol negative feedback is necessary for pubertal onset (Ramirez and McCann, 1963). According to this concept, decreased sensitivity to steroid negative feedback allows increased pituitary gonadotropin secretion which subsequently results in ovarian follicle maturation and ovulation.

The "gonadostat" theory has been tested in ewes and female rats. Physiological concentrations of exogenous estradiol suppressed luteinizing hormone (LH) to nondetectable concentrations in 19-wk-old ovariectomized ewe lambs (Foster and Ryan, 1979). In the same study, sensitivity of LH to estradiol negative feedback in these lambs decreased, coincidental with puberty in age-matched intact ewes. Peripubertal female rats, on the other hand, showed no major decrease in sensitivity to estradiol feedback until after the first preovulatory LH surge (Andrews et al., 1981). The authors suggested that decreased sensitivity to estradiol negative feedback was a consequence, rather than a controlling mechanism of pubertal onset in the female rat. Therefore, certain concepts of the "gonadostat" theory may not be applicable to all species.

Available data indicate that many individual components of the reproductive endocrine system in the heifer are operational before estrous cycles are initiated. For example, prepubertal heifers respond to exogenous gonadotropin-releasing hormone (Barnes et al., 1980) and to the positive feedback effects of estradiol (Schillo et al., 1981) with LH surges similar to or greater than those elicited by these treatments in mature females. Fertile ovulations can also be induced by 1 mo of age in heifers using exogenous gonadotropins (Seidel et al., 1971). However, limited information is available regarding the endocrine mechanisms that regulate the pubertal process in heifers. Injections of estradiol which resulted in supraphysiological serum estradiol concentrations suppressed LH secretion at 4, 8 and 12 mo of age in ovariectomized heifers (Schillo et al., 1982). The duration of suppression was longer at 4, than 8 or 12 mo of age, suggesting that a change in sensitivity to estradiol negative feedback may occur during sexual maturation in heifers.

The major objectives of the present experiments were: a) to evaluate the responsiveness of LH secretion in prepubertal heifers to estradiol negative feedback and b) to determine if changes in estradiol negative feedback on LH secretion occur during sexual maturation in heifers. An additional objective was to characterize LH secretion in intact, prepubertal heifers. Parts of these data have been presented briefly elsewhere (Day et al., 1981, 1982).

MATERIALS AND METHODS

Experiment 1

Seven prepubertal Angus X Hereford heifers were used in this experiment. All heifers were placed on a diet restricted in energy at 289 ± 10 (X ± SEM) days of age when body weight was 195 ± 6 kg. Intake, as well as energy content of the feed was restricted. This diet, formulated for body weight gains of 150 g/head daily was used to delay onset of puberty (Gonzalez-Padilla et al., 1975) and was fed until initiation of the experiment when the heifers age and body weight were 508 ± 10 days and 235 ± 6 kg, respectively (Day 0). A blood sample was collected from each heifer on Days −16 and −6. These samples were assayed for progesterone concentration to ensure that heifers were anestrous prior to Day 0. Six heifers were ovariectomized via high lumbar laparotomy on Day 0 and visual examination of ovaries indicated that estrous cycles had not been initiated. Three of the ovariectomized heifers received no additional treatment (OVX) while the remaining 3 ovariectomized heifers were administered a subcutaneous polydimethylsiloxane implant (3.35 mm, i.d. X 4.65 mm, o.d. X 270 mm; Dow-

Corning, Midland, MI) filled with estradiol-17β (Sigma Chemical Co., St. Louis, MO) caudal to the shoulder blade on Day 0 (OVX-E_2). The seventh heifer was used as an intact control (CONT). Dietary energy levels were gradually increased (Day 0 to Day 12), until heifers were consuming a diet formulated for gains of 0.91 kg/head daily. This diet was fed for the remainder of the experiment. Maintenance of heifers on a low-energy diet delayed puberty and the subsequent feeding of a high-energy diet reduced the variation in age at puberty (Gonzalez-Padilla et al., 1975).

Sequential blood samples were collected by jugular venipuncture at 12-min intervals for 8 h before ovariectomy on Day 0 and again on Days 4, 16, 30, 44, 58, 72, 86 and 100. These samples were used to evaluate mean concentrations of serum LH and the patterns of LH secretion. Single blood samples were collected from the CONT heifer at 3-day intervals and assayed for progesterone to estimate the time of puberty. All blood samples were allowed to clot at room temperature and then stored at 4°C. Blood was centrifuged within 48 h of collection at 1520 X g for 15 min at 4°C. Serum was removed and frozen at −20°C until assayed. Heifers remained docile throughout the collection periods and precautions were taken to avoid stress throughout the study.

Experiment 2

A second more extensive experiment using a similar experimental design was performed with 16 prepubertal Red Angus X Hereford heifers. Dietary energy restriction was not imposed in this study and heifers were allowed to reach puberty spontaneously. This study was initiated when the heifers age and body weight were 266 ± 3 days and 177 ± 4 kg, respectively (Day 0). This age and weight was selected for initiation of the experiment based primarily upon the observed age and weight at puberty of heifers with similar breeding in previous years. At this age and weight, all heifers were expected to be prepubertal, however, onset of puberty was expected to occur within 150 days after initiation of the experiment. Heifers assigned to OVX and OVX-E_2 treatments were ovariectomized on Day 0 (266 ± 3 days of age: 177 ± 4 kg body weight). OVX-E_2 heifers received an implant identical to that used in Experiment 1 on Day 0. The experiment was designed to continue until all CONT heifers reached puberty. During the experimental period heifers were fed a diet formulated for weight gains of 0.91 kg/head daily.

Sequential blood samples were collected at 12-min intervals for 8 h prior to ovariectomy on day 0 and again on Days 8, 36, 50, 64, 78, 92, 106, 134, 148, 162 and 176 for determination of basal and episodic LH secretion. Single blood samples were collected from CONT heifers at 7-day intervals and assayed for progesterone to estimate the time of puberty. Blood samples were processed as indicated in Experiment 1.

Polydimethysiloxane implants have been shown to release constant quantities of estradiol over time (Dziuk and Cook, 1966). However, during the treatment period, body weight of all heifers and presumably blood volumes were increasing. Therefore some reduction in serum concentrations of estradiol were expected with increased body weight in OVX-E_2 heifers, but the total amount of estradiol received each day during the study by OVX-E_2 heifers via the implant would

not change. A second identical implant was administered to OVX-E_2 heifers on Day 164 after all CONT heifers had reached puberty to determine if changes in LH secretion that occurred in OVX-E_2 heifers during the prepubertal period were physiological, or simply a result of diluted serum estradiol concentrations. Serum LH concentrations were determined in sequential samples (12-min intervals for 8 h) collected 2 days before, and 12 days following, administration of the second estradiol implant.

Radioimmunoassays

Serum LH concentrations were quantified by radioimmunoassay as validated by Golter et al. (1973). LH samples were analyzed in 37 assays with 280 samples (duplicate 200-μl aliquots) per assay. Each assay contained heifers from all treatments, distributed as equally as possible, from a single or adjacent bleeding day. Intra- and interassay coefficients of variation were 3.1% and 11.7%, respectively. Assay sensitivity was 264 pg/ml.

Serum estradiol concentrations were measured in pools of 10 serum samples collected during sequential sampling periods by the methods of D'Occhio et al. (1982). Estradiol concentrations were analyzed in 7 assays containing 15 to 25 samples (duplicate 500-μl aliquots). Heifers from all treatments were represented in every assay, and all samples analyzed for an individual heifer were contained in a single assay in order to minimize the effect of interassay variation on treatment differences. Intra- and interassay coefficients of variation were 1.9% and 19.4%, respectively. The limit of detection for estradiol assays was 1.40 pg/ml of serum.

Serum progesterone concentrations were analyzed for CONT heifers by radioimmunoassay (Anthony et al., 1981). Intra- and interassay coefficients of variation were 1.5% and 6.4%, respectively. The limit of detection for progesterone assays was 25 pg/ml of serum (duplicate 200-μl aliquots).

Data Analyses

Mean serum LH concentration (ng/ml), LH pulse frequency (pulses/8 h), and LH pulse amplitude (ng) for individual heifers during each period of sequential blood collection were calculated. Mean serum LH concentrations for individual heifers on days of sequential blood collection were calculated as the average of the 40 samples collected during each 8-h period. LH pulse frequency for individual heifers was calculated as the sum of all pulses detected during each 8-h period of blood collection. Criteria used to identify pulses were as follows: 1) the peak had to occur within 2 samples of the preceding nadir, 2) the amplitude had to be greater than the assay sensitivity, and 3) the LH concentration at the peak had to exceed the 95% confidence limits of the preceding and following nadir (Goodman and Karsch, 1980). LH pulse amplitude for individual heifers on days of sequential blood collection was calculated as the average amplitude of all pulses detected during each 8-h period. Amplitude was calculated as the concentration at the peak minus the concentration at the preceding nadir (Goodman and Karsch, 1980).

In both experiments, the day of puberty was defined as the first day that serum progesterone concentrations indicated the presence of a corpus luteum that was functional for the duration of a normal estrous cycle, followed by subsequent profiles of progesterone secretion indicative of normal estrous cycles. In Experiment 1, blood samples were collected every 3 days from the CONT heifer and analyzed for progesterone concentration. The criterion for identification of the first normal estrous cycle with this sampling interval was that serum progesterone concentrations had to be maintained above 1 ng/ml for at least 3 samples. The criteria for Experiment 2, when samples were collected every 7 days were: 1) serum progesterone concentrations had to be sustained above 1 ng/ml for at least 2 samples; or 2) serum progesterone concentrations had to be greater than 2 ng/ml for at least 1 sample. In both experiments, normal estrous cycles, as defined with the above criteria, had to occur immediately after the pubertal estrous cycle for determination of the day of puberty. Estrous cycles followed by periods of anestrus did not occur in either experiment. Short luteal phases normally observed in prepubertal heifers (Gonzalez-Padilla et al., 1975; Berardinelli et al., 1979) were detected in some of the intact heifers but the above criteria excluded these short rises in serum progesterone concentrations from calculation of the day of puberty. The above criteria were developed based upon the concentrations of progesterone detected with our assay system during the estrous cycle in heifers (Imakawa et al., 1983).

The day when estradiol negative feedback ceased to inhibit LH secretion ("0 inhibition") in OVX-E_2 heifers in Experiment 2 was calculated. This day was identified as the day that mean serum LH concentration or LH pulse frequency in each individual OVX-E_2 heifer attained or surpassed "castrate" levels of LH secretion. "Castrate" levels of LH secretion were defined as the mean serum LH concentration and LH pulse frequency detected in OVX heifers 36 days following ovariectomy at which time it appeared that the maximal acute response to ovariectomy had occurred. A similar pulse frequency but lower mean LH concentrations were detected in acute (30 days) or chronic (>12 mo) mature ovariectomized heifers (Kinder et al., 1983). Calculation of the day of "0 inhibition" was performed to determine a physiological end point for OVX-E_2 heifers that could be compared to the physiological end point of puberty for age-matched CONT heifers.

Data for CONT heifers were standardized to the day of puberty in order to characterize LH secretion in intact heifers as puberty approached. Data for OVX-E_2 heifers were standardized to the day of "0 inhibition" and were compared to LH secretion as puberty approached in CONT heifers. This comparison was performed only for Experiment 2, as data for only 1 CONT heifer were available in Experiment 1.

In both experiments, data were analyzed using standard analysis of variance procedures for split-plot, completely randomized design with treatment as the whole plot and days as the split plot (Steele and Torrie, 1980). Orthogonal contrasts were used to test differences between treatments. Specific mean comparisons were performed using the Fisher-protected least significant difference test (Steele and Torrie, 1980). Changes in LH secretion over time were characterized using regression analysis (Draper and Smith, 1966). Spline regression analysis was performed if dis-

TABLE 1. Age and body weight[a] at various stages of pubertal development in heifers.

Stage	Experiment 1		Experiment 2	
	Age (days)	Weight (kg)	Age (days)	Weight (kg)
Weaning[b]	189 ± 10	180 ± 4	163 ± 3	105 ± 4
Initiation of diet restriction[c]	289 ± 10	195 ± 6	---	---
Day 0[d]	508 ± 10	235 ± 6	266 ± 3	177 ± 4
Puberty[e]	578	306	384 ± 10	283 ± 10
"0 Inhibition"[f]	563 ± 17	311 ± 10	395 ± 10	308 ± 29

[a]Mean ± SEM.

[b]Experiment 1 (n=7); Experiment 2 (n=16).

[c]Experiment 1 only (n=7); diet formulated for gains of 150 gm/head daily.

[d]Day of ovariectomy, or ovariectomy and estradiol implant administration in respective treatments in both experiments and day of initiation of the high-energy diet in Experiment 1.

[e]Day of first increase in progesterone indicating a normal corpus luteum. Experiment 1 (n=1); Experiment 2 (n=6).

[f]Day when LH secretion in ovariectomized, estradiol-implanted heifers was equal to or greater than that detected in ovariectomized heifers. Experiment 1 (n=3); Experiment 2 (n=5).

tinctly different phases of LH secretion appeared to exist within one or more treatments (Draper and Smith, 1966). Since only 1 CONT heifer was used in Experiment 1, all statistical analyses included only OVX and OVX-E$_2$ treatments. In Experiment 2, CONT heifers were removed from the analyses after the onset of puberty. Five of 6 CONT heifers had attained puberty before the period of sequential blood collection on Day 148, therefore analyses were performed only to Day 134, at which time 3 CONT heifers remained in the prepubertal state.

RESULTS

Body Weights

Body weight changes in both experiments are shown in Table 1. Body weight on Day 0

and weight changes throughout each experiment were similar among treatments.

Serum Estradiol Concentrations

Estradiol concentrations for both experiments are shown in Table 2. Concentrations on Day 0 were similar for all treatments. Estradiol concentrations were nondetectable (P<0.01) in OVX heifers by Day 44 in Experiment 1 and by Day 8 in Experiment 2. Serum estradiol concentrations were increased (P<0.01) above pretreatment levels in OVX-E$_2$ heifers by Day 4 in Experiment 1 and Day 8 in Experiment 2. Average estradiol concentrations detected over the

TABLE 2. Mean serum estradiol concentrations (pg/ml) in intact, ovariectomized, and ovariectomized, estradiol-implanted heifers.

Treatment	Days relative to ovariectomy											
	Experiment 1						Experiment 2					
	0	4	44	58	100	Mean	0	8	50	106	134	Mean
CONT[a]	5.8	6.6	3.9	4.2	---	5.18	6.8	4.9	5.0	4.2	7.4	5.66
OVX[b]	6.5	4.3	ND[d]	ND	ND	2.16	6.0	ND	ND	ND	ND	1.20
OVX-E$_2$[c]	7.3	13.1	7.2	5.3	7.5	8.08	5.5	9.9	9.3	7.4	5.3	7.48

[a]Intact prepubertal heifers: Experiment 1 (n=1); Experiment 2 (n=6).

[b]Ovariectomized prepubertal heifers: Experiment 1 (n=3); Experiment 2 (n=5).

[c]Ovariectomized, estradiol-implanted prepubertal heifers: Experiment 1 (n=3); Experiment 2 (n=5).

[d]ND=nondetectable concentrations.

treatment period were higher (P<0.05) in OVX-E$_2$ than in CONT heifers in Experiment 2.

Patterns of LH Secretion for Individual Heifers

Patterns of LH secretion for a representative heifer from each treatment in both experiments are shown in Fig. 1. These profiles indicate changes in mean LH concentration, pulse frequency and pulse amplitude for individual animals, and are provided as a reference to illustrate the temporal changes in group means discussed in the following results.

Pubertal Response of LH Secretion to Estradiol

Mean LH concentration and LH pulse frequency increased (P<0.01) rapidly (Days 0 to 30, Experiment 1; Days 0 to 36, Experiment 2) following ovariectomy in OVX heifers (Figs. 2 and 3). Mean serum LH concentrations in OVX heifers stabilized after this abrupt increase, but LH pulse frequency continued to increase (P<0.05) in a more gradual, linear manner (Days 44 to 100, Experiment 1; Days 50 to 134, Experiment 2). The acute response of LH secretion to ovariectomy was blocked by estradiol in OVX-E$_2$ heifers (Days 0 to 30, Experiment 1; Days 0 to 36, Experiment 2).

Changes in Response of LH Secretion to Estradiol Negative Feedback

Experiment 1. LH secretion was essentially nonpulsatile and serum LH concentrations were basal from Days 0 to 44 in OVX-E$_2$ heifers (Figs. 2 and 3). An abrupt 5- to 7-fold increase (P<0.01) in mean serum LH concentration and LH pulse frequency occurred in all OVX-E$_2$ heifers between Days 44 and 58. Representative changes in pulse amplitude at this time can be observed in Fig. 1. A single, large pulse of LH occurred in the CONT heifer on Day 44 and puberty occurred on Day 60.

Experiment 2. Mean serum LH concentration and LH pulse frequency increased (P<0.01) gradually from Days 0 to 134 in OVX-E$_2$ and CONT heifers (Figs. 2 and 3). During this time period, mean LH concentration and LH pulse frequency increased at different rates in OVX-E$_2$ than in CONT heifers. LH pulse amplitude was variable during this time and can be observed for representative heifers in Fig. 1. Puberty in CONT heifers occurred on Day 122 ± 10 (Figs.

2 and 3). Castrate levels of LH secretion ("0 inhibition") occurred on Day 128 ± 25 in OVX-E$_2$ heifers. Age and weight at puberty in CONT heifers were similar to age and weight at "0 inhibition" in OVX-E$_2$ heifers (Table 1).

The second estradiol implant, administered to OVX-E$_2$ heifers on Day 164 increased (P<0.01) serum estradiol concentrations from 5.56 ± 1.0 pg/ml on Day 162 to 12.35 ± 1.6 pg/ml on Day 176. Mean serum LH concentration, LH pulse frequency and LH pulse amplitude on Day 162 were not different than those detected on Day 176.

Characterization of Prepubertal LH Secretion

Table 3 indicates prepubertal changes in LH secretion for CONT heifers in Experiment 2 when data were standardized to the day of puberty rather than to the day of initiation of the experiment. Mean LH secretion and LH pulse frequency increased (P<0.001) in a gradual linear manner during the 126 days prior to the onset of puberty. Mean LH concentration and LH pulse frequency increased (P<0.001) as "0 inhibition" approached in OVX-E$_2$ but at a different rate (P<0.05) than detected in CONT heifers as puberty approached.

DISCUSSION

Ovariectomized heifers with supplemental exogenous estradiol were used as the model animal to evaluate the relationships of estradiol feedback and LH secretion during pubertal development in the present experiments. The concentrations of serum estradiol resulting from the estradiol implants were slightly higher than those detected in the intact prepubertal heifers. However, these concentrations were similar to those detected during diestrus and less than those detected at estrus in heifers (Henricks et al., 1974), indicating that they were within the normal physiological range of estradiol concentrations present in the bovine female.

Exogenous estradiol completely inhibited the acute postcastration increase in LH secretion in ovariectomized prepubertal heifers in both experiments. In contrast, Beck et al. (1976) reported that elevation of serum estradiol to 20 to 30 pg/ml in ovariectomized postpubertal heifers did not completely suppress the acute postcastration increase of LH secretion. Additional data from our laboratory (Kinder et al., 1983) indicate that administration of one or
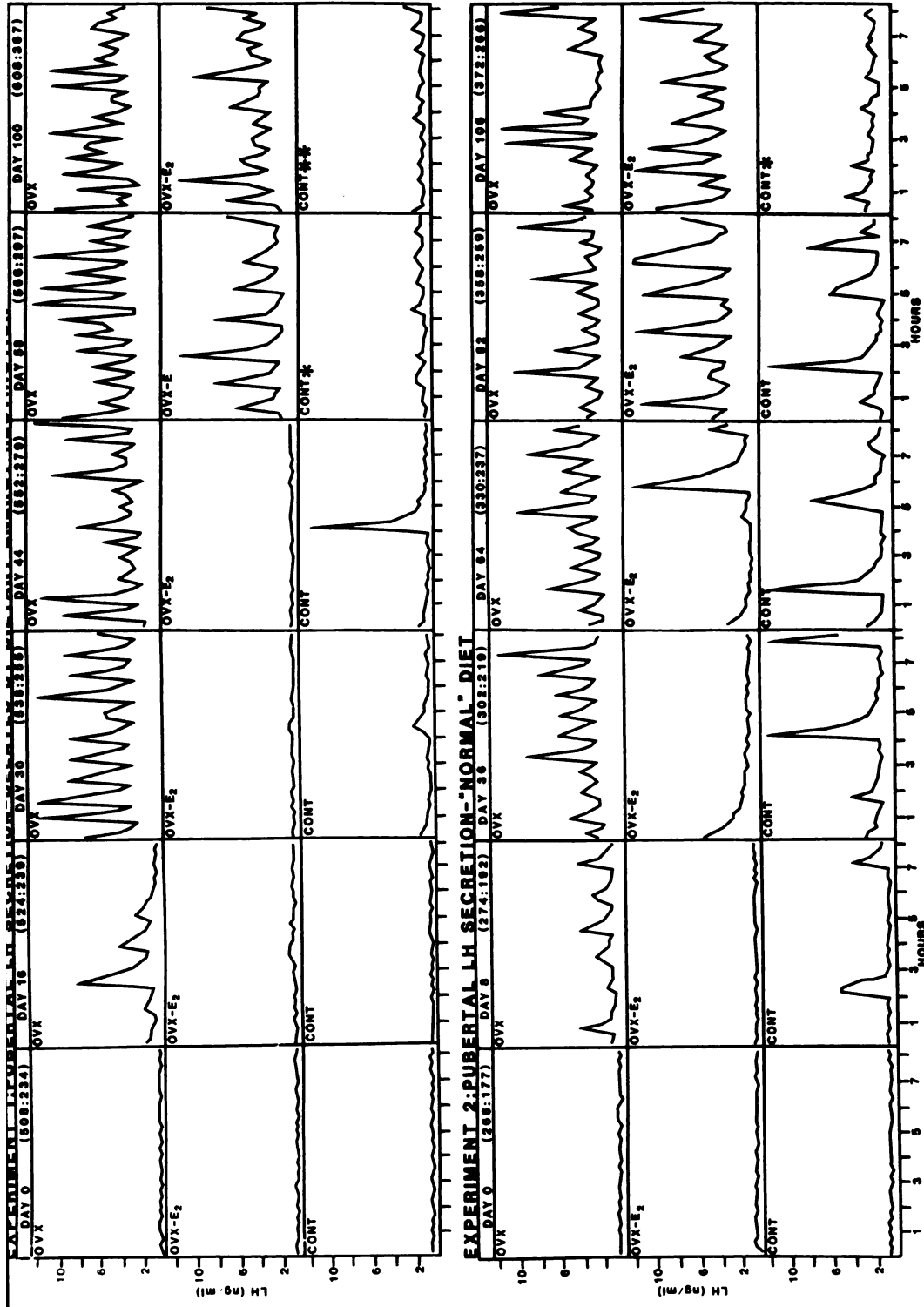
FIG. 1. Patterns of LH secretion during 8-h sequential blood collections of representative heifers in each treatment for Experiments 1 and 2. *Numbers in parentheses* represent mean age (days); body weight (kg) for all heifers on that day. *=Proestrous prior to first ovulation in CONT heifers; **=proestrous of third estrous cycle in CONT heifer.

two implants identical to those used in the present experiments enhanced rather than suppressed LH secretion in mature ovariecto- mized heifers 30 days following implantation. Results of this study and other data demon- strate that LH secretion is more sensitive to es- tradiol negative feedback in prepubertal heifers than in mature heifers. This relationship is con- sistent with previous observations in female rats (Eldridge et al., 1974; Andrews et al., 1981) and ewe lambs (Foster and Ryan, 1979).

In Experiment 2, LH secretion increased gradually in ovariectomized heifers implanted with estradiol when data were standardized to the day of "0 inhibition." Castrate levels were attained at the same age and weight at which puberty occurred in the intact heifers. In Experiment 1, LH secretion was low in ovariec- tomized, estradiol-implanted heifers prior to the marked increase which occurred coinciden- tal with onset of puberty in the intact heifer. These results indicate that the negative feedback of estradiol on LH secretion decreases during pubertal development in heifers. It appears that estradiol feedback decreased in an abrupt manner when reproductive state was synchron- ized by dietary manipulation, whereas changes were more gradual in heifers that attained puberty spontaneously. The synchrony of puberty in the intact heifers with attainment of castrate levels of LH in ovariectomized, estradiol- implanted heifers suggest that decreased estra- diol negative feedback on LH secretion is im- portant for endocrine regulation of puberty in heifers. Decreased feedback of estradiol on LH secretion has also been reported to function in the pubertal process of ewe lambs (Foster and Ryan, 1979) but it may not be a controlling mechanism of puberty in female rats (Andrews et al., 1981).

The possibility that the results obtained were due to dilution of estradiol as a result of weight gains during both experiments or to the possible development of refractoriness of the hypothalamo-hypopyseal centers which regulate LH secretion to estradiol negative feedback must be addressed. In reference to the question of dilution, the results obtained after adminis- tration of the second implant in Experiment 2 indicate that this was not the case. The second implant caused a 65% increase in serum estradiol to concentrations greater than were detected at any other time during the study. However, no suppression of elevated LH secretion was detected. The possibility of refractoriness is

also minimal. Decreased feedback occurred simultaneously in all three ovariectomized, estradiol-implanted heifers between Days 44 and 58 in Experiment 2, whereas castrate levels were not obtained until Day 128 in Experiment 2, with considerable between animal variation. The synchrony of response in Experiment 1 suggests a physiological phenomenon, as this type of dietary treatment has been shown to synchronize puberty (Gonzalez-Padilla et al., 1975). The variation in Experiment 2 was expected, as considerable variation in age at puberty was detected in the intact heifers. If refractoriness was the cause of decreased estradiol feedback, a similar time from implant administration to increased LH would have been expected in all heifers and the difference in timing and variation in decreased LH inhibi- tion would not have occurred.

Schams et al. (1981) observed a biphasic profile of LH secretion for prepubertal Brown Swiss heifers. Mean plasma LH concentrations increased from birth to 90 days of age, then decreased to a nadir at 180 days of age. Values increased again from 180 to 270 days of age and puberty was detected at 300 days of age. Frequency of LH pulses appeared to increase gradually from birth to puberty. In the male, Lacroix and Pelletier (1979) concluded that increased frequency of episodic LH pulses occurs coincidental with the onset of testicular development in bull calves. Pulsatile LH secre- tion is necessary for normal testicular growth and spermatogenesis in bull calves (Schanbacher, 1981; Schanbacher et al., 1982). In the present experiments, mean serum LH concentration and the frequency of episodic LH pulses increased gradually in intact heifers (Experiment 2) during the 126 days prior to puberty. The increased mean LH concentrations appeared to be at least partially due to the increased fre- quency of LH pulses and perhaps to an increased basal level of LH. These results indicate that LH secretion increases as puberty approaches in heifers and suggest a role of increased episodic and/or mean circulating concentrations of LH for pubertal development in heifers. Ryan and Foster (1978) reported an increase in mean LH concentrations 2 to 6 days before onset of puberty in ewe lambs, although this finding was not substantiated in a later study (Foster and Ryan, 1979). No peripubertal increase in LH secretion was detected until the day of the first preovulatory surge in female rats by Ojeda et al. (1976). However, more recent research
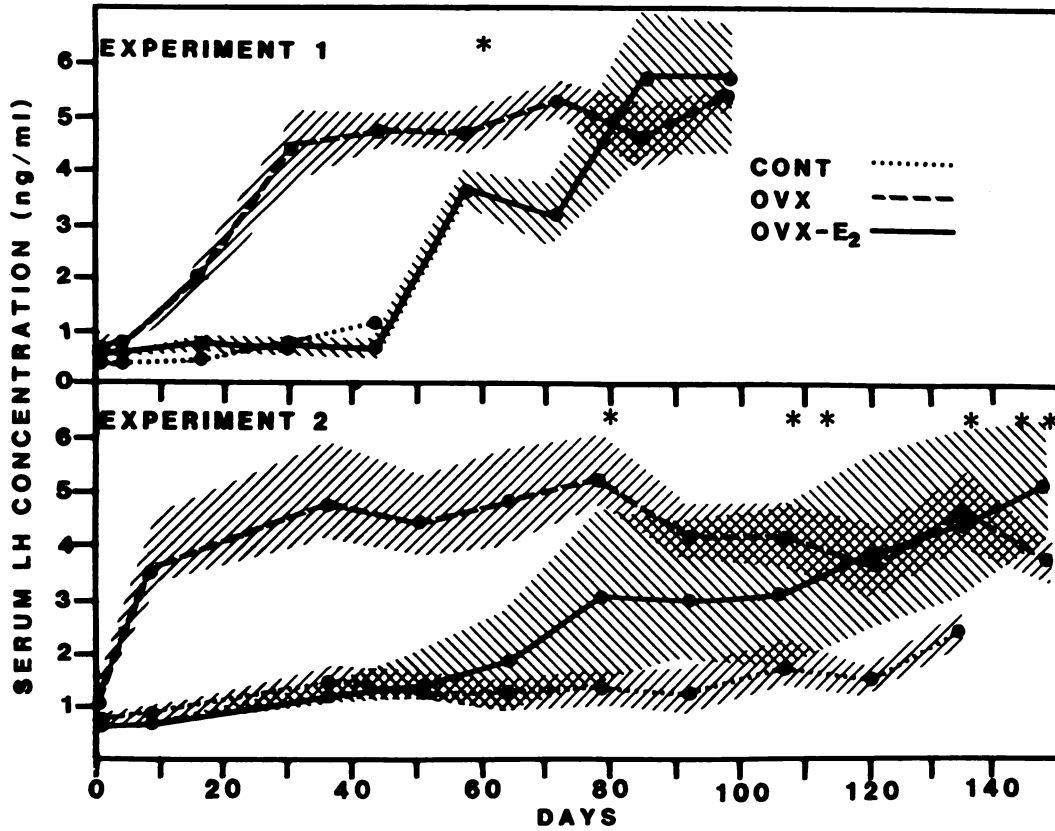
FIG. 2. Mean ± SEM (*shaded area*) serum LH concentrations for intact (*CONT*) ovariectomized (*OVX*) and ovariectomized, estradiol-implanted (OVX-E_2) heifers in Experiments 1 and 2. *=Indicates time of puberty for each CONT heifer.

TABLE 3. Mean serum in concentration (ng/ml) and LH pulse frequency (pulses/h) prior to puberty in intact heifers.

Day[a]	Mean LH concentration[b]	LH pulse frequency[c]
−126	0.85 ± 0.26	0.04 ± 0.04
−112	1.22 ± 0.29	0.06 ± 0.06
− 98	0.93 ± 0.20	0.13 ± 0.06
− 84	1.11 ± 0.25	0.15 ± 0.05
− 70	1.33 ± 0.30	0.19 ± 0.07
− 56	1.16 ± 0.23	0.19 ± 0.08
− 42	1.48 ± 0.29	0.25 ± 0.08
− 28	1.48 ± 0.24	0.31 ± 0.05
− 14	2.30 ± 0.20	0.48 ± 0.10

[a]Days relative to puberty.

[b]$Y=0.53 + 0.15x$; $P>0=0.0001$, $R^2=0.28$.

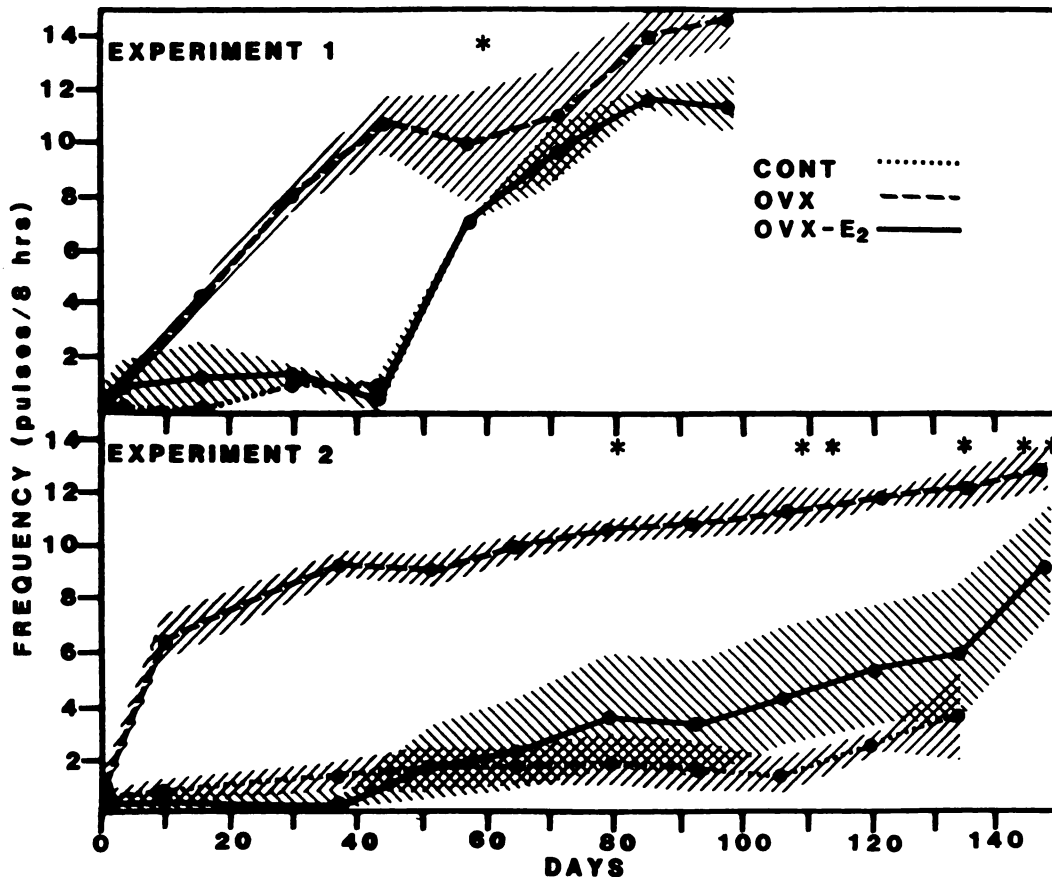[c]$Y=0.36 + 0.38x$; $P>0=0.0001$, $R^2=0.36$.

FIG. 3. Mean ± SEM (*shaded area*) LH pulse frequency for intact (*CONT*), ovariectomied (*OVX*) and ovariectomized, estradiol-implanted (*OVX-E₂*) heifers. *=Indicates time of puberty for each CONT heifer.

indicates that mean serum LH concentrations increase during the 2 wk preceding puberty in female rats (Meijs-Roelofs et al., 1983). Species differences in prepubertal LH release may exist, however, it is possible that the subtleness of the changes occurring can only be detected by intensive blood collection procedures similar to those used in the present experiments.

The results of this research suggest that the onset of puberty in the heifer is, at least in part, a result of decreased estradiol negative feedback on LH secretion. The probability that additional mechanisms exist which act in concert with decreased estradiol feedback to control pubertal development is likely, and is an area that warrants further investigation.

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