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ENDOCRINE CHANGES DURING RESTORATION OF ESTROUS CYCLES FOLLOWING INDUCTION OF ANESTRUS BY RESTRICTED NUTRIENT INTAKE IN BEEF HEIFERS¹

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ABSTRACT

The working hypotheses in this experiment were: 1) that ovarian estradiol would inhibit luteinizing hormone (LH) secretion in heifers that were anestrus as a result of restricted dietary energy intake and 2) the responsiveness of LH secretion to estradiol negative feedback would decrease during the period when restoration of estrous cycles occurred following feeding of diets adequate in energy. Fifteen heifers weighing 341 ± 12 (mean $\pm$ SE) kg were fed a diet containing 50% of the energy required for maintenance until 40 to 50 d following cessation of estrous cycles. Heifers were assigned to intact control (C, $n = 5$), ovariectomized (OVX, $n = 5$) or ovariectomized-estradiol-17 β -implanted (OVX + E₂, $n = 5$) treatments. Heifers were subsequently provided a high-energy (HE) diet until termination of the study. Progesterone concentrations indicating cessation of corpus luteum function were detected after heifers had lost 71 ± 8 kg body weight over 186 ± 28 d. Control heifers re-initiated estrous cycles as indicated by increased progesterone concentrations in serum at 49 ± 9 d after initiation of feeding the HE diet (360 ± 18 kg body weight). Initiation of pulsatile LH secretion was observed in heifers by d 12 following OVX. Estradiol suppressed LH secretion in OVX + E₂ heifers during the period of nutritional anestrus in C heifers. Suppressive effects of E₂ on LH secretion continued in OVX heifers after C heifers had initiated corpus luteum function. Therefore, the working hypothesis that LH secretion is inhibited by E₂ in the nutritionally anestrous heifer is accepted but responsiveness to estradiol does not subside with re-initiation of estrous cycles, thus this working hypothesis is rejected.

(Key Words: LH, Anestrus, Energy, Heifers, Estrous Cycle.)

Introduction

Secretion of hormones in the bovine female can be modulated by the nutritional regimen (Hill et al., 1970; Gombe and Hansel, 1973; Beal et al., 1978; Imakawa et al., 1983). Anes-

trus has been evoked by limiting energy intake in heifers, and estrous cycles were re-established when diets adequate in energy were provided following the period of dietary energy restriction (Bond et al., 1958).

Increases in luteinizing hormone (LH) secretion are prevented following ovariectomy (OVX) in prepubertal lambs (Foster and Ryan, 1979) and heifers (Day et al., 1984) implanted with estradiol-17 β (E₂). As heifers mature, responsiveness of the hypothalamo-hypophyseal axis to E₂ negative feedback decreases (Schillo et al., 1982; Day et al., 1984). The time of decreased responsiveness to E₂ in OVX heifers treated with E₂ coincides with the onset of puberty in intact heifers (Day et al., 1984). A similar influence of E₂ on LH secretion has been observed in seasonally anestrous ewes (Legan 1977) and postpartum beef cows that are lactationally anestrus (Acosta et al., 1983). These data indicate that the effect of E₂ on LH secre-

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tion is similar in sexually immature, seasonally and lactationally anestrus animals.

The present study was undertaken to determine if E_2 inhibited LH secretion in nutritionally induced anestrus heifers, and to evaluate the effects of E_2 on LH secretion during the re-establishment of estrous cycles after the diet deficient in energy was replaced with a diet containing adequate levels of energy.

Materials and Methods

Experimental Animals and Estradiol Implants. Fifteen nulliparous bovine females (March born; Angus $\times$ Hereford), 20 mo of age at the start of the experiment (November 17, 1980), were used in this study. These post-pubertal heifers weighed 341 ± 12 kg (mean $\pm$ SE) when the study began. Heifers from this herd normally reach puberty at 12 mo of age (270 kg body weight) and weigh about 500 kg when mature at 4 yr of age. They were maintained outdoors and were fed as a group in two .3-ha paddocks. All heifers were weighed before the time of feeding on two consecutive days at 2-wk intervals throughout the study; the average weight of the two measurements was used to determine weight changes. Careful observations of health were made daily at each feeding period. Ovariectomy (OVX, day of OVX = d 0) was performed via high lumbar laparotomy using local anesthesia (Lidocaine hydrochloride⁴, 2%). The silastic capsule⁵ (id = 3.35 mm, od = 4.65 mm 27-cm length) containing crystalline E_2 was inserted sc caudal to the shoulder blade at the time of OVX. The implant remained in place throughout the experimental period. In other experiments this type of silastic implant containing estradiol has remained in situ for 2 yr without a detectable decrease in concentrations of E_2 in peripheral serum (M. L. Day and J. E. Kinder, unpublished data).

Experimental Design. Before initiation of feeding restricted levels of dietary energy, estrous cycles were synchronized in all heifers by using 25-mg prostaglandin $F_{2\alpha}$ ⁶ ($PGF_{2\alpha}$) injections 10 d apart. Serum progesterone concentrations were quantified daily for 21 d to ensure that a normal luteal phase occurred after the

second $PGF_{2\alpha}$ injection. Serum progesterone concentrations greater than 1 ng/ml were considered indicative of luteal function (Humphrey et al., 1983). The feeding of a diet (sun-cured alfalfa pellets; $8.8 \text{ Mcal} \cdot \text{head}^{-1} \cdot \text{d}^{-1}$) providing 50% of the energy required for maintenance as recommended by the National Research Council (NRC, 1976) was initiated 21 d subsequent to the second $PGF_{2\alpha}$ injection. Trace mineralized salt and water were provided ad libitum. Corpus luteum activity was determined by quantifying peripheral progesterone concentrations in samples collected once weekly throughout the study. Feed restriction was terminated when serum progesterone concentrations were less than 1 ng/ml for a 40- to 50-d period. Heifers were assigned at random to either intact control (C, $n=5$), ovariectomized (OVX, $n=5$) or ovariectomized-estradiol-implanted (OVX + E_2 , $n=5$) treatments at this time. Therefore, the heifers had been anestrus for 40 to 50 d at the time of OVX. All OVX heifers were shifted to a diet containing higher energy levels immediately following OVX. Thus, initiation of feeding the higher energy diet to C heifers occurred after they had been anestrus for 40 to 50 d. The higher energy diet (HE diet) contained alfalfa hay, corn and oats ($22.4 \text{ Mcal} \cdot \text{head}^{-1} \cdot \text{d}^{-1}$) and was fed until termination of the study.

Serial blood samples were collected by jugular venipuncture at 12-min intervals for 6-h periods on d -1, 12, 26 and at 2-wk intervals thereafter to determine secretory patterns of LH. Estradiol concentrations were determined in pools from the first 10 samples of each serial blood collection. Serum was harvested and stored at -20°C until assayed. The experiment was designed to terminate at the time estrous cycles resumed in all C heifers.

Hormone Analyses. Serum LH concentrations were determined by the double antibody radioimmunoassay as described by Golter et al. (1973), in all of the serial samples using rabbit antiserum against bovine LH (JJR-RABLH#5), highly purified iodinated ovine LH (LER-1056-C2) as labelled hormone and NIH-LH-B7 as standard. Serum LH has been determined in 100-, 200- and 300- μl sample volumes in each of three pools of bovine serum in our laboratory. Assay determinations were parallel to the standard curve (pool 1 = 2.83, 2.73 and 2.70; pool 2 = 2.20, 2.13 and 1.83; and pool 3 = 1.60, 1.23 and 1.22 for 100, 200 and 300 μl serum, respectively). Added mass equivalent to the lowest six values of the standard curve was

⁴Med-Tech, Inc., Elwood, KS 66024.

⁵Dow-Corning Corp., Midland, MI 48640.

⁶Lutalyse, Upjohn Company, Kalamazoo, MI 49001.

added at 3 vol (20, 50 and 100 μ l) of each of three sera pools. Only assay values of less than the highest value (2 ng/tube) were used to validate the assay. Regression of deviations from expected values of eight of nine combinations revealed no trends ($P > .05$) toward over-estimation or under-estimation of the recovery of added mass (Neter and Wasserman, 1974). The ninth combination revealed no trend ($P > .10$) toward over-estimation or under-estimation. The sensitivity for the LH assay was .32 ng/ml. Intra- and inter-assay coefficients of variation for LH assays were 3.8 and 11.2%, respectively. The progesterone assay used was validated by Anthony et al. (1981). The assay sensitivity for progesterone was .16 ng/ml. Intra- and inter-assay coefficients of variation were 4.4 and 13.6%, respectively. Serum E_2 concentrations were quantified by pooling the serial samples and using the method described by D'Occhio et al. (1982). The assay sensitivity for E_2 was determined to be 1.76 pg/ml. Intra- and inter-assay coefficients of variation were 4.5 and 12.9%, respectively.

Statistical Analyses. Methods of Goodman and Karsch (1980) were used to identify LH pulses in data obtained from serial blood samples. The criteria for determining if a pulse of LH had occurred were: 1) a peak had to occur within two samples of the previous nadir, 2) the amplitude had to be greater than the sensitivity of the assay and 3) the level of LH at the peak had to exceed the 95% confidence limits of the concentrations at both the preceding and subsequent nadir. Confidence limits and assay sensitivity were determined using the methods of Duddleson et al. (1972). Amplitude of pulses of LH was defined as the concentration of LH at the peak minus that at the previous nadir. Serum concentrations of LH and amplitude of the LH pulses were averaged during each serial collection period. After heifers in the C group resumed estrous cycles, subsequent samples from C heifers were excluded from data analyses.

Analysis of variance for a split-plot design was used to test for differences in concentrations of LH, number of LH pulses, amplitude of LH pulses and E_2 concentrations (Steel and Torrie, 1980). Orthogonal contrasts were performed to assess differences among treatments. Changes in secretion of LH over time were characterized using regression analysis (Draper and Smith, 1981).

Results

Concentrations of progesterone indicative of the onset of anestrus were first detected at 186 ± 28 d ($n = 15$) following the initiation of feeding the diet restricted in energy. At this time heifers had lost 71 ± 8 kg (20% of initial weight; $n = 15$) body weight after initiation of feeding the diet restricted in energy. In four of five heifers in the C group, progesterone concentrations indicative of the onset of corpus luteum function and thus the re-initiation of estrous cycles occurred 41 ± 5 d after resumption of feeding the HE diet. The body weight of the heifers at this time was slightly heavier than the initial weight. Re-initiation of the estrous cycle did not occur until d 82 in the remaining C heifer. This heifer lost 25% of her body weight before the onset of anestrus. All heifers in the C group ($n = 5$) re-initiated estrous cycles by 49 ± 9 d.

Mean concentrations of serum LH in C heifers increased gradually ($P < .05$) to re-initiation of estrous cycles (figure 1). Ovariectomy caused marked increases in concentrations of serum LH ($P < .01$) in heifers from d -1 to 40; no further increase ($P > .15$) in LH occurred thereafter. Estradiol suppressed mean concentrations of LH in OVX heifers during the period following anestrus when they were fed the HE diet. Suppressive effects of E_2 on LH secretion in OVX heifers did not subside concomitantly with the re-initiation of estrous cycles in C heifers.

Figure 1. Mean ($\pm$ SE, shaded area) concentrations of serum LH in heifers during the period when a diet adequate in energy was being fed following the period of nutritionally induced anestrus.

Figure 2. Mean ($\pm$ SE, shaded area) LH pulse frequency (upper panel) and LH pulse amplitude (lower panel) in heifers during the period when a diet adequate in energy was being fed following nutritionally induced anestrus.

Gradual increases in frequency and amplitude of LH pulses were observed in C heifers following initiation of feeding the HE diet (figure 2). Frequency of LH pulses in OVX heifers increased linearly ($P < .01$), whereas amplitude of LH pulses increased ($P < .05$) abruptly (d -1 to 12), but subsequent changes were not observed during the study. There were no LH pulses detected in OVX + E₂ heifers during the period from d -1 to 54. After it was recognized that initiation of LH pulses in OVX + E₂ heifers was delayed, collection of samples for determination of LH secretion in three of them was continued until the initiation of LH pulses. Initiation of LH pulses in these heifers occurred 152 ± 35 d after resumption of feeding the HE diet. At this time these heifers weighed 452 ± 53 kg (figure 3).

Similar concentrations of E₂ were detected in all heifers on d -1 (table 1). Ovariectomy resulted in decreased concentrations of serum E₂ ($P < .05$) by d 12. Concentrations of serum E₂ in OVX + E₂ heifers were slightly elevated above pre-treatment concentrations on d 12. The only time when concentrations of serum E₂ differed in C and OVX + E₂ heifers was on d 54.

Discussion

Ovariectomy in heifers that were anestrus as a result of restricted dietary energy consumption resulted in an abrupt increase in secretion of LH during the period when estrous cycle restoration was occurring in C heifers. Root and Russ (1972) reported that gonadal removal resulted in similar concentrations of pituitary and/or serum LH and follicle stimulating hormone (FSH) in rats that received diets restricted in energy and in rats fed ad libitum. The data from the present study agree with the previous observation in that the pituitary in the nutritionally induced, anestrus heifer has the ability to secrete LH in high concentrations following gonadal removal.

Estradiol alone failed to suppress LH secretion in OVX heifers that were cyclic at OVX whereas a combination of E₂ and progesterone suppressed LH secretion (Beck et al., 1976). In OVX heifers fed adequate energy and cycling before OVX, concentrations of serum LH were higher in E₂-implanted heifers and identical to those used in the present study, when compared with LH in heifers not implanted with E₂ (Kinder et al., 1983). Crister et al. (1983) found that E₂ administered via silastic implants enhanced secretion of LH throughout the 12-mo period the implants were in situ in OVX heifers that were cyclic at the time of OVX. These data indicate that E₂ enhances LH secretion when administered via implants in post-pubertal heifers that were in adequate body condition. Day et al. (1986) has provided evidence that E₂ suppresses LH secretion below the levels of OVX

Figure 3. Mean body weight at times when changes in endocrine states occurred during periods of restricted and adequate feeding of dietary energy of heifers.

TABLE 1. MEAN CONCENTRATIONS (PG/ML) OF SERUM ESTRADIOL DURING THE RESTORATION PERIOD FROM NUTRITIONAL ANESTRUS^a

Treatment	Days post-ovariectomy ^b				
	-1	12	26	40	54
C ^c	3.55 ± .64	2.74 ± .93 ^f	2.28 ± .30 ^f	2.25 ± .64 ^f	2.54 ± .86 ^f
OVX ^d	2.84 ± .36	1.05 ± .51 ^g	1.45 ± .59 ^g	1.67 ± .37 ^g	2.15 ± .33
OVX + E ₂ ^c	2.79 ± .36	3.49 ± .41 ^f	3.32 ± .69 ^f	3.43 ± .48 ^f	4.03 ± .26 ^g

^aMean ± SE.^bOVX was performed 1 d before the initiation of feeding a diet high in energy.^cControl heifers that remained intact throughout study.^dOVX heifers.^eOVX-estradiol-17β (E₂) implanted heifers. E₂ implant was inserted sc at OVX.^{f,g}Means in the same column with unlike superscripts differ (P<.05) except d 40 post-OVX (P<.08).

heifers before the age when puberty would have been expected had they remained intact. Near the time when puberty would have been expected to occur in the OVX heifers implanted with E₂, there was a marked increase in LH secretion and thereafter the OVX heifers implanted with E₂ had higher concentrations of serum LH than those without E₂. In the present study the anestrus condition apparently caused the heifers to revert to the condition where E₂ suppressed LH below that of OVX heifers.

The abrupt increase in LH secretion in OVX heifers not implanted with E₂ in both the present and previous studies did not occur in OVX + E₂ heifers in the present study. The E₂ suppressed the secretion of LH in the nutritionally anestrus heifers in the present study similar to that observed in sexually immature (Foster and Ryan, 1979; Day et al. 1986), seasonally (Legan et al., 1977) and lactationally (Acosta et al., 1983) anestrus animals.

Estradiol appears to suppress secretion of LH by reducing the frequency of LH pulses in anestrus animals. The low frequency of LH pulses in anestrus animals appears to be due to a lack of hypothalamic stimulation by LH-releasing hormone (LHRH) rather than to the inability of the pituitary to respond to hypothalamic secretion (Goodman et al., 1982; McLeod et al., 1984). The inhibition of LHRH secretion in these animals could be due to the negative feedback effects of E₂ that inhibit synthesis and/or secretion of LHRH. Thus, the frequency of secretion of LH pulses is low in sexually immature, seasonally anestrus and lactationally

anestrus animals.

The frequency of LH pulses is thought to be insufficient for ovulatory follicle development, pre-ovulatory E₂ rise and ovulation in seasonally anestrus ewes (Goodman et al., 1982). This theory was supported by the studies of McNatty et al. (1981) and McNeilly et al. (1982) in which production of a follicular phase frequency of pulses of LH was accomplished by exogenous administration of LHRH to anestrus ewes. This resulted in a normal E₂ rise, LH surge and ovulation. When responsiveness to E₂ negative feedback subsides during the transition from seasonal anestrus to the breeding season, sustained increases in LH and E₂ become possible, thus enabling E₂ to reach the threshold for eliciting the pre-ovulatory surge of LH and allowing for re-establishment of estrous cycles (Karsch et al., 1980).

The suppressive effects of exogenous E₂ on LH secretion subsided in animals that were ovariectomized during the prepubertal or seasonal anestrus state coincidental with the onset of estrous cycles in intact animals (Legan et al., 1977; Foster and Ryan, 1979; Day et al., 1984). If decreased responsiveness of secretion of LH to the inhibitory feedback of E₂ was the only factor controlling LH secretion during the period of dietary energy restoration in the nutritionally induced anestrus heifers in the present study, the suppressive effects of E₂ on LH secretion in the OVX + E₂ heifers should have subsided coincidental to the time when estrous cycles were reinitiated in C heifers. From the results presented here, it cannot be determined

why the heifers implanted with E_2 did not overcome the feedback inhibition on LH secretion when C heifers re-initiated estrous cycles.

The results from the present study support the initial component of the working hypothesis that LH secretion is inhibited by E_2 in the nutritionally anestrus heifer. However, the second component of the hypothesis that inhibition of LH secretion by E_2 subsides with the onset of estrous cycles cannot be incorporated into a model for nutritional regulation of the reproductive endocrine system in cattle without further study.

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