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PLASMA LUTEINIZING HORMONE (LH) AND PROGESTERONE LEVELS IN HEIFERS ON RESTRICTED ENERGY INTAKES[1]

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Summary

A REVERSAL type experiment was conducted in which measurements were made of plasma levels of luteinizing hormone (LH) and progesterone in six Holstein heifers during three estrous cycles when they were maintained on either 100% or 62% of Morrison's TDN allowances. A progressive increase in plasma LH was noted from the first to third estrous cycle in heifers on the low energy intake. This increase was first seen in the estrous peaks, but by the third cycle the basal mean for the low TDN group (2.7 ng/ml) was also significantly higher than that of the control heifers (1.9 ng/ml, $P<0.005$). During the first cycle, plasma progesterone was slightly higher in the low calorie group, but became progressively lower in the subsequent cycles ($P<0.05$ and $P<0.01$). Both total progesterone and progesterone concentration in corpora lutea taken on the 10th day of the third cycle were lower in the low-TDN heifers than in their normal counterparts, $P<0.01$ and <0.05, respectivley. The results show that ovarian hypofunction under conditions of restricted energy intake is not due to reduced circulating levels of LH, as was previously hypothesized, and further suggest that the first effect is a reduced ability of the ovarian tissue to respond to LH.

Introduction

Mulinos and Pomerantz (1940) referred to the influence of undernutrition in reducing ovarian function as "pseudohypophysectomy". In 1947, Maddock and Heller provided the first critical evidence for the pseudohypophysectomy theory when they demonstrated that aqueous pituitary extracts of starved and control rats had equal gonadotropin potencies in restoring ovarian function of starved test rats. In separate reviews dealing with nutritional effects on hormone production, Leathem (1966) and Lamming (1966) advanced a pseudohypophysectomy theory as a possible explanation for the ovarian hypofunction seen under low nutrition. Until recently, there have been no simultaneous measurements on plasma gonadotropins and steroids in animals under various forms of food restriction. Hill *et al.* (1970) found a decrease in plasma progesterone within 5 days in cattle receiving 85% of maintenance requirements for energy and protein. No changes were noted in plasma luteinizing hormone during the single estrous cycles studied. Donaldson, Basset and Thorburn (1970), in studies with cattle, and Cumming *et al.* (1971), in studies of pregnant ewes, noted small, statistically significant rises in plasma progesterone, followed by significant declines in progesterone values in animals restricted to 25% of the total food intake of controls.

In our studies, simultaneous measurements of plasma LH and progesterone were made over three estrous cycles in order to determine if a decrease in plasma LH precedes ovarian hypofunction under the influence of restricted total digestible nutrient (TDN) intakes. Progesterone content and concentrations in corpora lutea (CL) removed during the third estrous cycle was also measured.

Materials and Methods

Animals. Six Holstein heifers weighing between 320 and 442 kg were kept in a dairy barn, except when turned into a paved yard at least three times daily, between 7 and 8 am, 12 and 1 pm and 5 and 6 pm for drinking

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water and heat detection. Animals were considered in estrus when they stood while being mounted by other heifers. Each heifer was examined over three cycles to ensure regularity of cyclic activity before being assigned to the experiment. A reversal experiment was carried out, in which three heifers were maintained on either 100% of Morrison's (1956) TDN allowances, or on 62% of these allowances. After the heifers had completed three estrous cycles on treatment, they were all fed on corn silage and hay *ad libitum* for 1 month, following which the groups were reversed for an additional three estrous cycles. The experimental rations fed are shown in table 1. The protein content of the two rations was adjusted by substituting soybean meal for corn in the low TDN group, so that the protein intake of the two groups was essentially equal.

During the second phase of the experiment, corpora lutea were harvested by intravaginal incisions on the 10th day of the third cycle, weighed and frozen until required for progesterone analyses.

The animals were fed individually each morning and routinely bled by jugular venipuncture at the time of feeding each day. Virtually all of the rations were consumed each day. Additional blood samples were collected at hourly intervals for 8 to 12 hr. beginning at the onset of estrus. The heparinized blood samples were kept in a refrigerator until centrifuged during the same day. The resultant plasma was kept frozen until assayed.

Hormones. Plasma LH was measured by the solid phase radioimmunoassay, as described by Hobson and Hansel (1972). The high-titer, TSH-absorbed antiserum used was prepared by Snook, Saatman and Hansel (1971) in a mare. Bovine LH preparations, NIH-LH-B7 and LER-1072-2 were originally supplied by the Endocrine Study Section of the National Institute of Health and by Dr. Leo E. Reichert, Jr. of Emory University, Atlanta, Georgia, respectively. The sensitivity of the assay has been discussed in detail by Hobson and Hansel (1972); the minimum amount of LH/assay tube that was significantly different from zero was 0.1 nanograms. All samples were assayed in triplicate. Three pools of known LH content were included in each assay. If variations of more than 0.2 ng/ml were found in any of these pools the assay was repeated; this occurred in only one case.

Plasma progesterone was measured by the protein binding assay of Murphy (1967), using male human plasma as a source of corticosterone binding globulin (CBG). Three milliliter replicates made basic by a few drops of concentrated ammonium hydroxide were extracted three times with reagent grade hexane. The combined supernates were dried *in vacuo* and stored in the cold until assayed. The standards were prepared at the same time and kept in the same way until used within the week. The CL were homogenized and lyophilized. Replicates of the resultant powder were extracted with ethanol and measured by successive thin layer and gas liquid chromatography, as described by Lukaszewska and Hansel (1970). Three pools of plasma, for which progesterone values had been determined by this thin layer-gas liquid chroma-

TABLE 1. DAILY RATIONS PROVIDED

Feed	Quantity (kg)	Estimated dry matter[a] (kg)	Estimated digestible protein[a] (kg)	Estimated TDN (kg)
(a) 100% TDN Group				
Mixed hay, good quality, less than 30% legume	7.3	6.5	0.33	3.5
Corn meal	1.8	1.6	0.13	1.5
Total	9.1	8.1	0.46	5.0
Requirement	...	8.4	0.45	4.5
(b) 62% TDN Group				
Hay, as above	4.5	4.0	0.20	2.2
Corn meal	0.45	0.40	0.03	0.38
Soybean meal	0.68	0.61	0.29	0.53
Total	5.6	5.0	0.52	3.11
Requirement	...	8.4	0.45	4.5

[a] Based on Morrison's (1956) tables.

tography method, were included in each plasma assay, and good agreement for the two methods was obtained.

Statistics. Analyses of plasma LH data were done on the basis of (a) values during estrus, (b) values from Day 4 before estrus to Day 5 after estrus, excluding the estrous peak, (c) combined estrus and basal values over the same period and (d) on basal values throughout the cycle, taking a mean cycle length of 20 days. Plasma progesterone values were analysed on the basis of values from Day 4 before estrus to Day 5 after estrus, and also on the basis of cycle values, taking, as before, a mean cycle length of 20 days. Plasma progesterone values in the cow decline rapidly at about Day 4 before estrus and do not rise appreciably until Day 5 after estrus (Hansel and Echternkamp, 1972). Progesterone contents and concentrations of CL were also compared. Least significant difference tests were performed on all sets of values showing significance on analysis of variance.

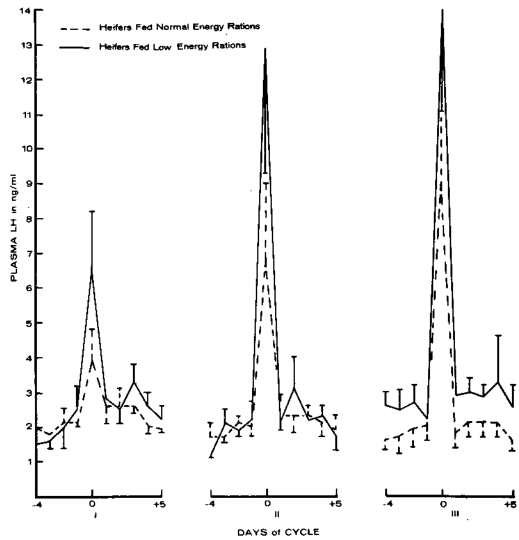

Figure 1. Plasma LH levels in Holstein heifers fed low and normal energy rations during three estrous cycles.

Results

The first and second cycles were of normal length in heifers on both rations. A progressive increase in plasma LH was noticeable, both in basal levels and peaks at estrus in the energy restricted heifers. Neither the basal, nor the estrous peaks, when considered alone, were significantly increased during the first and second cycles, (text figure 1) but taken together, the energy restricted group had higher mean plasma LH values, ±S.E.M., than the controls (3.3±0.4 ng/ml *vs.* 2.6±0.2 ng/ml for the first cycle and 3.2±0.3 ng/ml *vs.* 2.5±0.3 ng/ml for the second cycle, respectively, ($P<0.05$). By the third cycle the basal means of the low energy group were significantly higher than the control values (2.7±0.1 ng/ml *vs.* 1.9±0.1 ng/ml; $P<0.01$). When estrous and basal LH values were considered together for the third cycle, the values were 3.9±0.5 ng/ml for the energy restricted heifers *vs.* 2.6±0.4 ng/ml for the control animals; the differences were significant ($P<0.01$). Text figure 1 depicts the plasma LH values for Day −4 to Day +5 of each of the three estrous cycles studied. These means were calculated from 30 and 35 observations during the first cycle and 57 and 58 observations during the second cycle. The figures for the food restricted group are given first. During the third cycle the corresponding figures are 52 and 53 observations for basal LH values and 58 and 59 observations for both basal LH values and estrous LH peaks.

During the first cycle plasma progesterone values of the heifers on the restricted energy

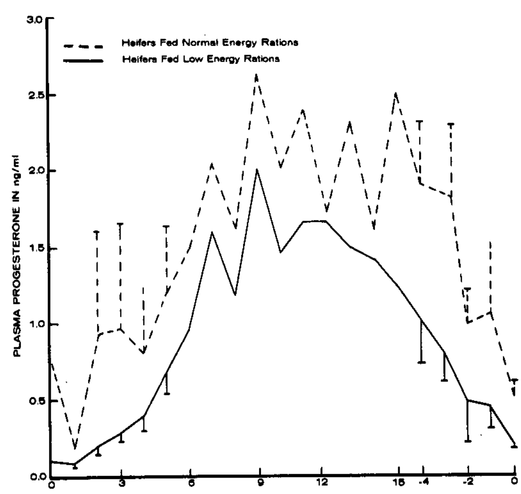

Figure 2. Plasma progesterone levels during the second estrous cycle in Holstein heifers fed low and normal energy rations for three estrous cycles.

intake were not significantly raised above the control animals (P>.05). However, during the second and third cycles the progesterone values of the underfed animals fell below the control values by 1–2 ng/ml (P<0.01 and P<0.05, respectively). These differences are depicted for the second estrous cycles in figure 2, and for the first 9 days of the third cycles in figure 3.

Significant differences were also found in CL removed on the 10th day of the third estrous cycle (figure 4). The heifers fed low energy rations had decidedly smaller corpora lutea than the controls, both in terms of wet and lyophilized weights ±S.E.M. (3.5±0.3 g *vs.* 6.4±0.4 g and 687.3±39.3 mg *vs.* 993.3±58.3 mg, respectively, P<0.01). Progesterone contents of the corpora lutea were also lower in the underfed heifers, 99.2±11.7 μg *vs.* 165.2±6.1 μg for total progesterone in the low calorie and control heifers, respectively (P<0.01). The differences in the concentrations of progesterone between the two groups, were significant (P<0.05; 138.4±8.1 μg/g dry weight *vs.* 168.1±3.6 μg/g dry weight for the energy restricted and normal heifers, respectively). In terms of wet weight, these concentrations translate to 27.6 μg/g and 33.6 μg/g for the energy restricted and control heifers, respectively.

Discussion

Hill *et al.* (1970) reported no change in basal plasma LH in beef heifers receiving

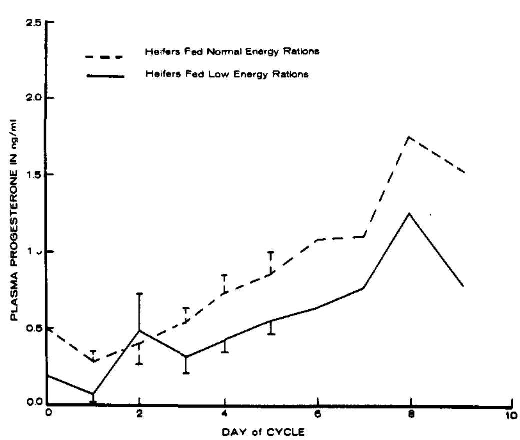

Figure 3. Plasma progesterone levels during the first 10 days of the third estrous cycle in Holstein heifers fed low and normal energy rations for three estrous cycles.

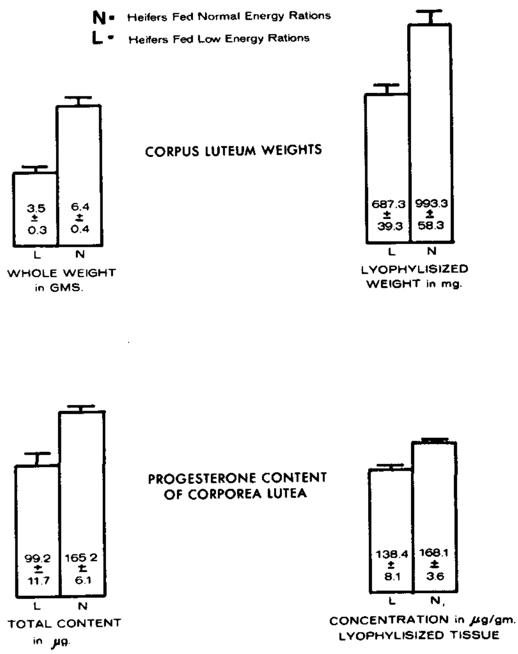

Figure 4. Weights and progesterone contents and concentrations in corpora lutea removed at the tenth day of the third estrous cycle in Holstein heifers fed low and normal energy rations for three estrous cycles.

85% of National Research Council requirements for energy and protein during one estrous cycle. However, their values did not include frequent LH measurements during estrus. In contrast, our data show that significant changes in plasma LH do occur during the second and third cycles when heifers are maintained on low energy intakes. However, the changes are not in the direction that would have been expected from the pseudohypophysectomy theory, since plasma LH in the energy-restricted animals was significantly elevated, rather than depressed. Thus, the decreased ovarian function seen under the influence of low energy intakes cannot be explained on the basis of reduced plasma LH, as appears to be the case in underfed rats (Meites, 1970; Howland, 1971, 1972).

Our data on plasma progesterone levels are in general agreement with those of Donaldson *et al.* (1970), who also reported significant decreases during the second and third estrous cycles in heifers receiving 25% of the total feed consumed by the controls. These workers found a significant increase in plasma progesterone levels during the first cycles; the increase observed during the first cycle in our experiment was not statistically significant. In contrast to these results, Hill *et al.* (1970) reported an immediate decline in plasma progesterone, which occurred within 5 days after the reduction of feed intake. Both protein and energy contents of the rations were varied in the studies of Donaldson *et al.* (1970) and Hill *et al.* (1970), while only the energy intake was reduced in our experiment. The reduction in plasma progesterone levels was evidently a reflection of the smaller CL, which contained less progesterone, both in terms of total content and concentration.

The simplest explanation of these somewhat unexpected results is that the first effect of restricted energy intake is at some step in steroidogenesis within the corpus luteum, causing the observed reduction in plasma progesterone. Since progesterone secreted by the corpus luteum appears to exert a negative feedback on LH secretion in the cow (Hobson and Hansel, 1972; Hansel and Echternkamp, 1972), the elevated LH levels observed may have been caused by the lowered plasma progesterone levels.

The apparent reduction in progesterone synthesis by the corpora lutea of the energy-restricted heifers could also have been due to a deficiency of a pituitary hormone other than LH that normally plays a role in corpus luteum maintenance. However, there is no clear-cut evidence that either FSH or prolactin has a luteotropic function in cattle (Hansel and Echternkamp, 1972). LH is known to be the major luteotropic hormone in the cow (Hansel, 1967), and it is thought to exert its effect by stimulating the conversion of a "steroidogenic" pool of cholesterol to pregnenolone (Wilks, Fuller and Hansel, 1970). The fact that progesterone secretion was depressed in the energy restricted animals, even in the presence of elevated plasma LH levels, suggests the possibility that dietary energy restriction may result in an inhibition of cholesterol side chain cleavage, since this is the point at which gonadotropins may act (Menon *et al.,* 1965; Major, Armstrong and Greep, 1967). LH causes an increase in adenyl cyclase activity and cyclic AMP formation in bovine luteal tissue (Marsh *et al.,* 1966) and the lowered progesterone synthesis in the energy restricted animals could be related to impairment of this important first step in LH action.

Since neither plasma estrogens nor FSH were measured in these studies, no assessment of the roles these hormones may have played in the results is possible. Estrogens are luteolytic in the cow (see Hansel and Echternkamp, 1972, for review), and it seems unlikely, in view of the rapid decrease in number and sizes of follicles noted in underfed heifers (Hill *et al.,* 1970), that the plasma levels of estrogens would have been elevated in the underfed animals.

Finally, the possibility must be considered that the lowered plasma progesterone and elevated plasma LH levels in the underfed heifers were due to some factor other than the restricted energy intake. Both rations contained the same amounts of digestible protein, but intakes of vitamins and minerals may have been different. However, severe vitamin or mineral deficiencies would not be expected to occur in animals on either ration during the time periods involved, and no gross evidence of such deficiencies was noted.

Literature Cited

Cumming, I. A., B. J. Mole, Jr. Obst, M. A. De B. Blockey, C. G. Winfield, and J. R. Goding. 1971. Increase in plasma progesterone caused by undernutrition during early pregnancy in the ewe. J. Reprod. Fertil. 24:146.

Donaldson, L. E., J. M. Basset and G. D. Thorburn. 1970. Peripheral plasma progesterone concentration of cows during puberty, oestrus cycles, pregnancy and lactation and the effects of undernutrition or exogenous oxytocin on progesterone concentration. J. Endocrinol. 48:599.

Hansel, W. 1967. Studies on the formation and maintenance of the corpus luteum. *In G. E.* Lamming and C. Amoroso (Ed.) Reproduction in The Female Mammal. Butterworths, London.

Hansel, W. and S. Echternkamp. 1972. Control of ovarian function in domestic animals. Amer. Zoologist 12:225.

Hill, J. R., D. R. Lamond, D. M. Henricks, J. F. Dickey and G. D. Niswender. 1970. The effects of undernutrition on ovarian function and fertility in beef heifers. Biol. Reprod. 2:78.

Hobson, W. C. and W. Hansel. 1972. Plasma LH levels after overiectomy, corpus luteum removal, and estradiol administration in cattle. Endocrinol. 91:185.

Howland, B. E. 1971. Gonadotropin levels in female rats subjected to restricted feed intake. J. Reprod. Fertil. 27:467.

Howland, B. E. 1972. Effect of restricted feed intake on LH levels in female rats. J. Anim. Sci. 34:445.

Lamming, G. E. 1966. Nutrition and Endocrine System. Nutr. Abstr. Rev. 36:1.

Leathem, J. H. 1966. Nutritional effects on hormone production. J. Anim. Sci. 25: Suppl. I. 68.

Lukaszewska, J. H. and W. Hansel. 1970. Extraction and partial purification of luteolytic activity from bovine endometrial tissue. Endocrinol. 86:261.

Maddock, W. O. and C. G. Heller. 1947. Dichotomy between hypophyseal content and amount of circulating gonadotropins during starvation. Soc. Exp. Biol. Med. 66:595.

Major, P. W., D. T. Armstrong and R. O. Greep. 1967. Effect of luteinizing hormone *in vivo* and *in vitro* on cholesterol conversion to progestins in rat corpus luteum tissue. Endocrinol. 81:19.

Marsh, J. M., R. W. Butcher, K. Savard and E. W. Sutherland. 1966. The stimulatory effect of luteinizing hormone on adenosine 3'5' monophosphate accumulation in corpus luteum slices. J. Biol. Chem. 241:5436.

Menon, K. M. J., M. Droskowsky, R. I. Forman and E. Forchielli. 1965. Side chain cleavage of cholesterol-26-^{14}C and 20 hydroxycholesterol-22-^{14}C by rat testes mitochondrial preparations and the effects of gonadotropin administration and hypophysectomy Steroids. Suppl. I. 95.

Meites, J. 1970. Modification of synthesis and release of hypothalamic releasing factors induced by exogenous stimuli. *In* L. Martini and J. Meites (Ed.) *Neurochemical Aspects of Hypothalamic Function.* Academic Press, New York and London. p. 1–36.

Morrison, F. B. 1956. Feeds and Feeding 22nd Ed. Morrison Publishing Co., Ithaca, New York.

Mulinos, M. G. and L. Pomerantz. 1940. "Pseudohypophysectomy". A condition resembling hypophysectomy produced by malnutrition. J. Nutr. 19:393.

Murphy, B. E. P. 1967. Some studies of the protein binding of steroids, their application to the routine micro- and ultramicro-measurement of various steroids in body fluids by competitive protein binding radioassay. J. Clin. Endocrinol. Met. 27:973.

Snook, R. B., R. Saatman and W. Hansel. 1971. Serum progesterone and luteinizing hormone levels during the bovine estrous cycle. Endocrinol. 88: 678.

Wilks, J. W., G. B. Fuller and W. Hansel. 1970. Role of cholesterol as a progestin precursor in rat, rabbit and bovine luteal tissue. Endocrinol. 88:581.