FERTILIZATION AND BLOOD LEVELS OF PROGESTERONE AND LH IN BEEF HEIFERS ON A RESTRICTED ENERGY DIET


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SUMMARY

Two groups of yearling beef heifers were fed different levels of nutrition to study the effect of reduced energy intake on fertilization rate and blood levels of progesterone and luteinizing hormone (LH). Control heifers received a ration meeting N.R.C. recommendations for all nutrients, while heifers on the restricted diet received only one-third of the recommended energy.

Ova recovery rates were 62 and 82%, respectively, for heifers fed low or high levels of energy. Eighty-eight percent of ova recovered from heifers on the low level of energy were fertilized compared to a 79% fertilization rate for ova recovered from heifers on the high level of energy. Neither of these differences were significant. No significant differences were found in blood levels of progesterone or LH either between nutrition group or among successive estrous cycles within nutrition group. No relationship could be established between actual weight change and blood levels of progesterone. Diet had no effect on number of follicles nor follicular or luteal volume. However, the ovary containing the corpus luteum was 57% larger in heifers fed adequate energy than in those restricted in energy intake (P<.05).

These results suggest that reduced pregnancy rate in heifers restricted in energy intake is not the result of fertilization failure, but some later causative factor. Determinations of levels of progesterone and LH in daily blood samples did not provide evidence to implicate any changes in these hormones that might indicate causes for embryonic loss.

(Key Words: Fertility, Progesterone, Luteinizing Hormone, Energy, Reproduction, Beef Cattle.)

INTRODUCTION

A positive relationship exists between weight gain and fertility in the bovine. Cows bred while gaining weight have a higher pregnancy rate than those bred while losing weight, and restricting energy intake after calving reduces pregnancy rate (Wiltbank et al., 1962, 1964; Turman et al., 1964; King, 1968; Schilling and England, 1968; Dunn et al., 1969; McClure, 1970). A portion of this reduction in pregnancy rate can be attributed to a decreased incidence of behavioral estrus. However, even if estrus is manifested, pregnancy rate appears to be decreased in cows losing weight.

The lowered pregnancy rate in undernourished cows may be the result of abnormal hormonal levels or patterns. In the first cycle on reduced energy intake, blood levels of progesterone in the cow have been reported to increase (Donaldson et al., 1970; Dunn et al., 1974), decrease (Hill et al., 1970), or remain the same (Gombe and Hansel, 1973). Effects of underfeeding for more than one cycle on systemic levels of progesterone are also disputed. Donaldson et al. (1970) and Gombe and Hansel (1973) reported reductions in serum levels of progesterone during successive cycles in cows on restricted energy intake. In contrast, Dunn et al. (1974) indicated that levels of progesterone in the bovine were consistently higher during the second cycle after a reduction in energy intake.

A definite reduction in blood levels of progesterone occurs in undernourished rats.
This reduction is the result of decreased gonadotropin secretion (Leatham, 1966; Howland, 1971, 1972; Howland and Ibrahim, 1971). The effects of restricting energy intake on concentration of luteinizing hormone (LH) in the bovine are unclear. Gombe and Hansel (1973) reported progressive increases in average levels of LH when heifers were on low levels of energy for three successive estrous cycles. However, Hill et al. (1970) and Dunn et al. (1974) reported no change in blood levels of LH during periods of restricted energy intake.

Hill et al. (1970) reported a reduction in fertilization rate in cows when energy intake was restricted. However, observations for fertilization were conducted at 3, 8 and 18 days postmating and may have overestimated fertilization failure. No reductions in fertilization rate were observed in ewes fed 25% of maintenance requirements for energy (Cumming et al., 1971).

The present study was conducted to determine if there is an effect of a reduction in energy intake on fertilization rate and blood levels of progesterone and LH in cycling beef heifers.

**MATERIALS AND METHODS**

Forty cycling heifers of predominantly Angus breeding, 15 to 19 months of age and averaging 334 kg (267 to 415 kg), were used in this study. Twenty heifers were initially fed a ration meeting National Research Council (N.R.C.) recommendations for all nutrients (high level) while the remaining 20 heifers were restricted to one-third of N.R.C. recommendations for energy, with all other nutrients fed to meet N.R.C. recommendations (low level) (N.R.C., 1964). A mixture of salt and dicalcium phosphate was available ad libitum. Heifers were weighed weekly and individual rations adjusted so that heifers receiving the high energy ration made a slight weight gain, while those on the low energy ration lost about .5 kg per head per day. Weights were recorded in early morning after overnight deprivation of both feed and water.

Heifers were fed in a fenceline bunk with feed placed directly in front of each heifer. Heifers were halted and chained to the bunk with approximately 3 ft of bunk space available per heifer. Due to limitations imposed by the lead chain, each heifer could consume only a limited amount of additional feed from adjacent animals. Heifers were tied and fed at approximately 1600 hr and they remained tethered until the evening estrus check at 1900 hours. Any uneaten feed was removed when heifers were untied. When not being fed or bled, heifers were in a dry-lot with free access to water. Estrous detection was conducted at 12-hr intervals by visually observing heifers for approximately 45 min for homosexual behavior.

As individual heifers came into estrus a local anesthetic was administered and a jugular vein cannulated with a 16-gauge indwelling catheter (Safedwell, Becton Dickinson). Two sutures secured the catheter to the skin over the jugular vein. Thirty-five milliliters of blood were collected daily from each heifer between 1400 and 1600 hours. The bleeding schedule continued until day 12 of the third estrous cycle or until an animal ceased to cycle. Blood samples were allowed to clot at room temperature for a minimum of 2 hr, stored at 5 C for 18 to 36 hr, and the serum harvested by centrifugation at 5 C. Serum samples were stored in plastic vials at −20 C until analyzed by the use of radioimmunoassay (RIA). Concentration of LH was measured with the RIA system described by Niswender et al. (1969). Concentration of progesterone was measured using the antisera prepared against progesterone-11α-BSA in the RIA system described by Niswender (1973).

Heifers on both nutritional regimes were bred at their second estrus. Heifers were artificially inseminated approximately 12 hr after being observed in estrus with frozen semen consisting of pooled ejaculates from three bulls. This semen pool has previously been shown to be of good fertility (Nelson et al., 1975). Ova were surgically recovered 48 to 96 hr after the heifer was first observed in estrus. Anesthesia was induced with Surital (Parke-Davis) and the animal was maintained in dorsal recumbency using a closed circuit system of halothane and oxygen. Surgical and ovum collection procedures were insignificant modifications of techniques reported by others (Rowson et al., 1969; Wishart and Young, 1974). Flushings were collected in 10 ml test tubes, transferred to watch glasses, and the presence of an ovum ascertained microscopically. Fertility was assessed by evidence of cleavage and presence of spermatozoa in the zona pellucida.

While the reproductive tract was exteriorized during surgery, ovarian dimensions and size of
The diameter of individual follicles and corpora lutea were measured with a mm ruler. The radius was taken as one-half of the diameter and substituted into the equation, \(4/3\pi r^3\) to calculate the volume of a sphere.

When the exact end of an estrous cycle was not known due to absence of observed estrus, it was estimated as 2 days after the level of progesterone had decreased to 50% of the value for the previous day. To compensate for differences in cycle length, data were normalized to the day of estrus (day 1), and daily means for the subsequent 11 days were plotted, followed by the 11 days prior to next estrus.

The data were subjected to standard analysis of variance to determine significance of differences noted in weight changes, ovarian size, follicular volume and luteal volume, while differences in fertilization rate were examined by chi-square procedures. Blood levels of LH and progesterone were evaluated by comparing the means by tests for means with equal or unequal number of observations and equal or unequal variances, depending on means being compared. Regression analysis was used to determine if a relationship existed between weight change and blood levels of progesterone (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Average weight changes were .023 and -.494 kg per head per day for heifers on high and low levels of energy, respectively (P<.01). After completing one estrous cycle on their respective nutritional regimes, 32 heifers were observed in estrus, inseminated, and laparotomized for ovum recovery (table 1). Two of 15 heifers on the low energy diet had not ovulated when ovum recovery was attempted. In each case, a follicle (10 and 16 mm, respectively) was found at surgery. One of these heifers was laparotomized again 2 days after the first surgery. Ovulation had occurred at the site of the previously noted follicle, but no ovum was recovered. An ovum was recovered from 62 and 82% of the ovulating heifers fed low and high energy diets, respectively (P>.05). The average fertilization rate of 82% observed in this study is in agreement with fertilization rates reported for the bovine (Kidder et al., 1954; Boyd et al., 1969). Eighty-eight percent and 79% of the ova recovered at 2 to 4 days postmating from heifers fed the low and high levels of energy, respectively, were fertilized (P>.05). The single ovum classified as unfertilized which was recovered from a heifer in the group on restricted energy had a ruptured zona pellucida containing spermatozoa. Since the contents of the zona pellucida were lost, it was impossible to accurately determine fertilization. No spermatozoa were found in the zona pellucida of the three unfertilized ova recovered from heifers on the high energy diet.

The ovulatory ovary in heifers on high energy intake was 57% larger than in heifers on restricted energy intake (P<.05, table 2). No significant differences among heifers on different diets were noted in either number of follicles or follicular or luteal volume. There was a trend for the corpus luteum of heifers fed the low level diet to be smaller. There is no obvious explanation for the difference in size observed in the ovulatory ovary, as the correlations between follicular volume and luteal volume with ovarian size were .175 and .173, respectively. In the non-ovulatory ovary, level of nutrition did not appear to have any significant effect on ovarian size or follicular volume.

Blood levels of progesterone (figure 1) and LH (figure 2) presented for cycle one are means of daily samples from 20 individual animals for both levels of energy. In cycle two the means are from daily samples on 17 heifers receiving

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of heifers</th>
<th>Heifers ovulated</th>
<th>Ova recovered</th>
<th>Fertilized ova</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heifers on high energy</td>
<td>17</td>
<td>17 (100%)</td>
<td>14 (82%)</td>
<td>11 (79%)</td>
</tr>
<tr>
<td>Heifers on low energy</td>
<td>15</td>
<td>13 (87%)</td>
<td>8 (62%)</td>
<td>7 (88%)^a</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>30 (94%)</td>
<td>22 (73%)</td>
<td>18 (82%)</td>
</tr>
</tbody>
</table>

^a An ovum in this group classified as unfertilized was recovered as a broken zona pellucida.
the high energy ration and 14 heifers receiving the low energy ration, and cycle three represents eight heifers from the high energy and six from the low energy group. In the high energy group three heifers stopped cycling (as suggested by lack of estrus and levels of progesterone), four were not observed in estrus and sampling stopped, and five were terminated for other reasons (illness or occluded catheter). Eight heifers on the low level of energy stopped cycling, three were not observed in estrus and three were terminated for other reasons. Mean cycle lengths for heifers completing the trial were 21.9 and 22.7 days for heifers on high energy and low energy diets, respectively (P > .05).

Level of nutrition had no significant effect, either between nutrition groups or among successive estrous cycles, on systemic blood levels of progesterone or LH over two and one-half estrous cycles. The observed levels of LH and progesterone were within the range of values reported in cycling cows and heifers (Henricks et al., 1971; Hansel and Echternkamp, 1972; Christensen et al., 1974; Gonzalez-Padilla et al., 1975). Concentration of LH remained at baseline levels of less than 1 ng/ml through the estrous cycle with elevations of 4 to 7 ng/ml on the day of estrus. Average levels of progesterone for both groups of heifers remained low, around .4 to .7 ng/ml for days 1 through 3 of the estrous cycle and began to increase on day 4, reaching values of 6 to 7 ng/ml during mid-cycle. This was followed by a precipitous decline 2 to 4 days prior to subsequent estrus. Since there were variations in average daily weight change among heifers within the two levels of energy, regression analysis was used to determine the relationship between average daily weight change and blood levels of progesterone over all animals in both nutrition groups. Neither a linear nor quadratic model, with weight change as the independent variable accounted significantly for the variability noted in blood levels of progesterone.

The results of this study are in disagreement with the reports of Donaldson et al. (1970) and Dunn et al. (1974) who showed increased systemic levels of progesterone in the bovine during the first cycle following reduction of energy intake. The data are also in conflict with those of Hill et al. (1970) who reported an immediate decrease in progesterone in serum within 5 days after initiation of restricted feeding. However, the results do support those
indicating that restricting energy intake has no immediate effect on concentration of progesterone in the cow (Gombe and Hansel, 1973). Our results are similar to data presented by Corah et al. (1974) who could detect no effect of prolonged undernutrition on systemic progesterone levels, and are in contrast to data indicating continued decline in serum levels of progesterone during successive estrous cycles on decreased energy intake (Donaldson et al., 1970).

The lack of a significant difference in blood levels of LH between heifers on the two levels of energy is in agreement with reports by Hill et al. (1970) and Dunn et al. (1974). However, these data are in conflict with those of Gombe and Hansel (1973) who reported progressive increases in mean systemic levels of LH during the first, second and third cycles for heifers restricted in energy intake.

Several explanations for the differences reported in blood levels of LH and progesterone for cows on restricted levels of feed are possible. Studies have been conducted under different environmental conditions, and there have been large differences in levels of energy in "restricted diets". Differences in age, breed, size and condition of the animals utilized in various studies could also cause differences in response. The small number of animals used in previous studies may also account for differences observed.

It would appear that the reduced pregnancy rate in cattle on restricted energy intake is not the result of fertilization failure, but is caused by embryonic loss after 4 days postmating. Determination of blood levels of progesterone and LH in daily samples did not provide evidence to indicate any changes in these hormones that would indicate causes for embryonic loss. The possibility exists that there would have been no difference in pregnancy rate between these two groups of heifers. However, in view of other reports, the rapid
weight loss immediately prior to and through mating in heifers on the low level of energy should ultimately result in decreased pregnancy rate (King, 1968; Schilling and England, 1968; McClure, 1970).

These results indicate a need for additional studies into the causes and time of embryonic loss in the bovine during periods of nutritional stress. If the time of embryonic loss in the bovine during periods of nutritional stress could be identified, more frequent blood sampling could possibly give some insight into the problems involved. Another area of importance may be the ability of the ovaries and uterus to respond to systemic levels of hormones, if no differences in absolute levels can be documented. At the present time a lack of information precludes any meaningful decisions as to the reasons for the lowered pregnancy rate observed in the underfed bovine. These data strongly suggest that neither fertilization failure nor average daily levels of LH or progesterone are involved.

LITERATURE CITED


