LUTEOTROPHIC FACTORS IN THE COW: EVIDENCE FOR LH RATHER THAN PROLACTIN

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Summary. The luteotrophic properties of endogenously secreted LH and prolactin were studied by measuring CL function after neutralization of circulating LH and prolactin by the administration of highly specific antisera against these hormones on the 11th and 12th day of the oestrous cycle. In addition, the effect of a prolactin inhibitor (CB-154), which reduced prolactin concentration in peripheral blood by 80 to 90%, was studied. A hysterectomized heifer carrying a persistent CL was treated with CB-154, a combination of CB-154 and prolactin antiserum and then with LH antiserum. In all animals treated with the LH antiserum, CL function ceased or was quantitatively inhibited. No change in circulating progesterone was seen after treatment with the prolactin antiserum or the prolactin inhibitor. It is concluded that LH is the dominant luteotrophic factor in the bovine species while prolactin has little or no activity.

INTRODUCTION

It has become evident in recent years that control of gonadal function is subject to considerable species variation. The luteotrophic action of LH or LH-like preparations in the cow, as indicated by increased progesterone production, has been demonstrated both in vivo and in vitro (Armstrong, Black & Cone, 1964; Marsh & Savard, 1964; Mason & Savard, 1964; Donaldson & Hansel, 1965; Armstrong & Black, 1966; Hansel, 1967; Schomberg, Coudert & Short, 1967; Seifart & Hansel, 1968; Hansel & Snook, 1970).

It has also been shown that endogenous LH has a rôle in maintaining CL function. Snook, Brunner, Saatman & Hansel (1969) found that treatment with a specific LH antiserum resulted in a reduction in the size and progesterone content of the bovine CL. Similarly, Karg, Hoffmann & Schams (1970) noticed a significant fall in the peripheral plasma concentration of progesterone after administration of LH antiserum on the 11th day of the cycle in one heifer. A less distinct effect was seen in a second animal.

The rôle of prolactin in the maintenance of CL function is not clear and the evidence is contradictory. It was originally believed, as a result of experiments in the rat, that prolactin was a luteotrophic hormone. Smith, McShan &
Casida (1957) and Donaldson, Hansel & Van Vleck (1965), however, could find no evidence of changes in the bovine oestrous cycle or in CL function after administration of prolactin. Bartosik, Romanoff, Watson & Scricco (1967), on the other hand, were able to demonstrate enhanced progesterone production during perfusion of bovine ovaries in vitro with prolactin.

In the present paper, a further attempt has been made to elucidate the rôle of LH and prolactin in the maintenance of CL function in cattle. Highly specific antisera against LH and prolactin and a specific prolactin inhibitor have been used in the normal cycling animal as well as in a hysterectomized heifer with a persistent functional CL.

MATERIALS AND METHODS

Animals

All animals used (heifers and cows) were of the Brown Swiss breed and had exhibited a normal reproductive pattern. One heifer was hysterectomized on the 11th day of the cycle to establish a persistent CL.

Hormone assays

Progesterone was determined by radioimmunoassay (RIA) as described by Hoffmann, Kyrein & Ender (1973). This method involves a simple plasma extraction with petroleum ether and employs an antiserum raised in rabbits against 11α-hydroxyprogesterone-hemisuccinate-bovine serum albumin.

The radioimmunological technique used for the determination of prolactin in bovine blood has been described in detail elsewhere (Schams & Karg, 1969). A reference preparation of NIH-P-B₂ was used as standard and the label was prepared using a preparation of bovine pituitary source. The separation of the antigen–antibody complex was performed by the double antibody method.

Antisera used for treatment

The antisera against LH and prolactin were obtained from rabbits and represented two pools from different bleedings. The LH antiserum was capable of binding 0·1 ng [¹²⁵I]LH at the 50% level when diluted 1:500,000. The antiserum at this dilution showed no cross-reaction with 200 ng prolactin (NIH-P-B₂). Reduction of binding equivalent to 0·4 ng LH was obtained with 6 ng purified ovine FSH (30 × the activity of NIH-FSH-S1), 120 ng growth hormone and 140 ng TSH. The antiserum raised against prolactin was used for radioimmunoassay purposes at a dilution of 1:200,000. At this dilution, it was able to bind 50% of 0·1 ng [¹²⁵I]prolactin. This antiserum showed no cross-reaction with 400 ng LH, 400 ng TSH, 400 ng purified sheep FSH (see above) or 400 ng growth hormone. The antisera were stored at −18°C until use when 30 ml were warmed to 20°C and infused intravenously over a period of 10 to 30 min.

Prolactin inhibitor

The prolactin inhibitor, CB-154 (2 Br α-ergokryptin-methane-sulphonate), was given subcutaneously as a single dose consisting of 100 mg dissolved in 1·0 ml 40% ethanol and then mixed with 2·0 ml propylene glycol.
**Experimental design**

*Experiment 1.* In five normally cycling animals (three heifers and two cows) progesterone was determined in a control cycle and in the following treatment cycle. The length of the cycle was defined as the period between two ovulations (Day 1 = the day of ovulation). Three of the animals (two heifers and one cow) were treated on the 11th and 12th days with LH antiserum. A total dosage of 90 ml antiserum was infused (2 × 30 ml on Day 11 and 1 × 30 ml on Day 12).

The fourth animal (Cow Ro) was treated in the same manner with prolactin antiserum. As a control, normal rabbit serum was administrated to the fifth animal (Heifer I). In the animals treated with LH antiserum or prolactin antiserum, the length of the six oestrous cycles preceding the treatment cycle was recorded individually for each animal.

*Experiment 2.* The prolactin inhibitor (CB-154) was given to two heifers (Z and B) as a single daily injection on Days 11 and 12 of the oestrous cycle. The progesterone concentrations were determined in the preceding control cycle and in the treated cycle.

*Experiment 3.* A hysterectomized heifer carrying a persistently functioning CL
was treated with 100 mg CB-154 on the 67th, 71st and 76th day after hysterectomy. On the 86th day, 100 mg CB-154 and 30 ml prolactin antiserum were given in the morning. This treatment was repeated on the following day in the morning and in the afternoon (only 24 ml antiserum could be given during the afternoon treatment). The animal was subsequently treated with 30 ml LH antiserum 138 days after hysterectomy and also on Day 139 in the morning and in the afternoon.

In all animals, peripheral plasma progesterone was measured as an index of CL function. In the experiments where only the prolactin inhibitor was administered, prolactin in peripheral plasma was also determined.

RESULTS

Experiment 1

When compared to the control cycle values, there was significant \( P<0.05 \) reduction in the circulating concentration of progesterone on Days 11 to 16 of the cycle following administration of LH antiserum (Text-fig. 1), but the quantitative effect was different in each of the three animals. Progesterone production was most affected in Heifer 5 and least affected in Cow Kf.

During and immediately after the infusion of prolactin antiserum, relatively low progesterone levels were observed but overall the progesterone secretion curve showed a similar pattern in the treatment and the control cycle (Text-fig. 2). Similarly, plain rabbit serum, given in a dosage of 30 ml on Days 11 and 12 of the cycle showed no effect on CL function (Text-fig. 2).
The length of the different oestrous cycles is given in Table 1. Only Cow Kf, which was treated with LH antiserum, showed any alteration of the cycle length, i.e. an increase to 25 days.

**Experiment 2**

As a result of the treatment, prolactin values in peripheral plasma were decreased by 83% and 88% in Heifers Z and B respectively. The average

![Graphs](https://via.placeholder.com/150)

**Text-fig. 3.** Progesterone values in the peripheral plasma of two heifers before and after the subcutaneous injection of a prolactin inhibitor (CB-154).

<table>
<thead>
<tr>
<th>Antiserum against</th>
<th>Animal</th>
<th>Duration of cycles (days)</th>
<th>Last six cycles before treatment</th>
<th>Treatment cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>Cow Kf</td>
<td>19 19 20 19 21 20</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heifer 6</td>
<td>21 22 20 20 19 22</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heifer 5</td>
<td>19 20 21 19 21</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Prolactin</td>
<td>Cow Ro</td>
<td>20 23 22 21 25 20</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>
Experiment 3

As can be seen from Text-fig. 4, CB-154 did not significantly alter CL function. This was also true for the time period when both the inhibitor and prolactin antiserum were used. The CB-154 alone caused a suppression of prolactin values in peripheral plasma by 45.6% from a mean of 2.4 ng/ml for the

![Text-fig. 4. Progesterone concentrations in peripheral plasma of a hysterectomized heifer (B) before and after treatment with a prolactin inhibitor (CB-154), the inhibitor in combination with prolactin antiserum and LH antiserum. Each point represents a mean of 5 days.](image)

![Text-fig. 5. Induction of luteal regression in a hysterectomized heifer after treatment with LH antiserum.](image)

last 15 days (Days 50 to 65) before the treatment to a mean of 1.3 ng/ml for the period from treatment to Day 80 after hysterectomy. Treatment with LH antiserum on Days 138 and 139, however, induced complete regression of the CL. As shown in Text-fig. 5, the first injection of antiserum was followed by an immediate drop in progesterone concentration from 6.0 ng/ml plasma to 1.7 ng/
ml plasma 13 hr later. Final luteolysis was induced by the second injection of LH antiserum, the progesterone values decreasing to less than 0.3 ng/ml plasma.

**DISCUSSION**

These experiments provide conclusive evidence that endogenous LH acts as the main luteotrophic factor in the cow. In each animal, the administration of LH antiserum led to regression of CL function. The antiserum used showed a high specificity towards LH. The small cross-reactivity observed with the growth hormone and TSH preparations was probably due to LH contamination of these two hormone preparations. The somewhat higher cross-reactivity observed with FSH is probably unimportant from the quantitative point of view and also when considering the biological activity of FSH. The control experiment in which normal rabbit serum was used confirms that the observations made concern a specific effect of the LH antiserum.

It is surprising that identical treatment with the LH antiserum yielded quantitatively different responses. In the hysterectomized animal, CL function virtually ceased while CL function in the three animals treated during the oestrous cycle was never completely suppressed. The effect was, however, very pronounced in one animal (Heifer 5) but not in a second animal (Cow Kf, Text-fig. 1). Two explanations could be given for this phenomenon. First, LH is not the only luteotrophic factor in the cow, but there are different supporting mechanisms of CL function in an intact animal and in a hysterectomized animal. The second and more likely possibility is that the neutralizing effect of the LH antiserum on the endogenously secreted LH is different in each animal (though the antibodies were given in excess, making LH analysis in blood plasma impossible for some time after treatment). The rate of destruction of the antibodies, individual elimination rates of the antibodies, individual secretion rates of LH and, perhaps most important, inhibition of LH at the cellular level are unknown and could all be subject to considerable variation between animals. The extension of the oestrous cycle in one animal (Cow Kf) was probably a result of the inhibition of the endogenous preovulatory LH peak, following administration of LH antiserum. This effect has already been described by Snook et al. (1969). The explanation is supported by the observation that it was principally the last phase of the cycle that was extended, following complete regression of the CL.

Our experiments indicate that prolactin has no luteotrophic properties in the cow. The relatively low progesterone concentrations in peripheral plasma determined in Heifer 4 (see Text-fig. 2) on Days 11 and 12 of the cycle during and immediately after treatment with prolactin antiserum do not seem to indicate an induced depression of CL function since a similar reduced progesterone production at about the same stage of the cycle was observed in the control cycle. As in the case of the treatment with LH antiserum, it must be assumed that the circulating endogenous prolactin was neutralized by the antiserum since the excess antibodies in the plasma precluded prolactin determination. Although it was not possible to measure changes in prolactin
levels in the experiments in which antiserum was used, an obvious decrease
was noted in the experiments with CB-154. The decrease was about 80 to 90% in
the animals treated during the cycle and around 46% in the hysterectomized
animal. The prolactin concentrations decreased to about the same value in all
three animals but relatively low resting values were found in the hysterectomized
animal as the experiments were performed in November. It is well known that
prolactin values in peripheral plasma of cattle show a pronounced seasonal
variation with lowest values in autumn and winter (Schams & Karg, 1970; 
Karg & Schams, 1970). Corpus luteum function did not appear to be affected
despite the observed reduction in circulating prolactin. This was true even for
the hysterectomized heifer with a persistent CL in which any long-term effects
of the prolactin inhibition on CL function should have become obvious. These
findings agree with the earlier observations of Hoffmann, Schams, Giménez,
Ender, Herrmann & Karg (1973) who found no change in progesterone
secretion after the administration of the inhibitor during the last 14 days of
pregnancy. In order to demonstrate complete elimination of prolactin from
peripheral plasma, the hysterectomized animal was treated with a combination
of inhibitor and prolactin antiserum. This treatment did not significantly alter
CL function.

Fluctuations in progesterone concentration which seemed to be greater
during and after the treatments became obvious because of the higher frequency
of blood collection compared to the control periods.

The experiments described have clearly shown, and have also confirmed
earlier reports, that LH is the primary luteotrophic factor in the bovine species.
A similar function cannot be attributed to prolactin for cattle on the basis on
these data.

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