SERUM PROLACTIN LEVELS IN CASTRATED RAMS AT VARIOUS TIMES OF THE YEAR AND DURING TREATMENT WITH ANDROGENS OR OESTROGEN

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SUMMARY

Prolactin concentrations were measured in serum from wethers at various times of the year before and during treatment with testosterone propionate, dihydrotestosterone propionate, 19-hydroxytestosterone dipropionate and oestradiol dipropionate. Levels of prolactin in serum were lower in untreated wethers during short (October) than during long (April–August) days. Seasonal differences persisted throughout the experiment but became less obvious during treatment with oestradiol dipropionate and 19-hydroxytestosterone dipropionate, both of which raised prolactin concentrations. Neither testosterone propionate nor dihydrotestosterone propionate altered the levels of prolactin.

INTRODUCTION

A positive relationship exists between the levels of prolactin in serum and daylength in intact and castrated rams kept under artificial photoperiods (Pelletier, 1973; Forbes, Driver, El Shahat, Boaz & Scanes, 1975; Lincoln, McNeilly & Cameron, 1978), and in intact rams reared under natural conditions (Ravault, 1976). However, the effect of season on prolactin concentrations in prepubertally castrated rams (wethers) has not been described. Moreover, the effects of exogenous gonadal steroids on prolactin levels in male sheep have not been investigated.

Administration of oestrogen or testosterone raises prolactin levels in castrated rats (Shin, Akin, Roberts & Howitt, 1974; Dohler, Wong & van zur Muhlen, 1978) and similar effects were produced with testosterone in juvenile monkeys (Herbert, 1978). However, the inability of treatment with dihydrotestosterone to influence prolactin concentrations in the castrated rat (Dohler et al. 1978) indicates that androgen aromatization may be necessary for the stimulation of prolactin secretion and/or release in the male rat. The possibility that a similar situation may exist in the male sheep was investigated in this study.

Serum prolactin levels were measured in wethers at various times of the year before and during treatment with propionated androgens or oestrogen. Two aromatizable androgens, testosterone and 19-hydroxytestosterone and one 5α-reduced androgen, dihydrotestosterone, were used.

METHODS

Twenty adult Clun Forest wethers were assigned to four equal groups. These groups were studied at different times of the year. The experimental period consisted of 2 weeks without
treatment (control period) followed by 6 weeks of daily s.c. injections (treatment period). Treatment commenced on the following dates for the four replicate groups: 18 April, 27 June, 5 September, 7 November, 1977. During the experimental period the sheep lived in a communal indoor pen illuminated by natural daylight; at other times they lived out in a field.

Individual sheep in each group were allocated different treatments for the duration of the 6 week period. The treatments were: oil vehicle; dihydrotestosterone propionate (20 mg/day); testosterone propionate (20 mg/day); 19-hydroxytestosterone dipropionate (20 mg/day); oestradiol dipropionate (2 mg/day).

To minimize any effects of animal handling or sampling order, the assay was based on the pooled blood from three jugular samples (10 ml) taken on the same day from each sheep. Samples were collected at hourly intervals from each animal every Tuesday afternoon throughout the 8 week experimental period. Blood was withdrawn into tubes containing a separation aid (Serum Monovette, Sarstedt Ltd, Leicester), it was allowed to stand for 20 min and then centrifuged. Serum samples were stored at −20 °C until the end of the experiment.

Serum samples were assayed using an antibody raised in rabbits against NIH-P-S11 ovine prolactin donated by NIH, Bethesda, Maryland. Iodination was carried out using the method of Thorell & Johansson (1971) but with the incubation time extended to 10 min. Assays were carried out at room temperature with 50 µl 125I-labelled hormone, 100 µl antisera (1 : 60 000 dilution) and 50 µl serum. The limit of detection was 12 ng ovine prolactin/ml and there was no cross-reaction with ovine growth hormone. Comparisons between groups and treatments were made by analysis of variance and Student’s t-test.

RESULTS

Levels of prolactin in the pretreatment period were significantly reduced during the short autumn photoperiods compared with the longer daylengths in spring and summer (Fig. 1).

The effects of the various hormone treatments on concentrations of prolactin are shown in Table 1; the standard errors are exaggerated due to the influence of the seasonal factors

![Fig. 1. Levels of prolactin (shaded bars; means ± S.E.M.) in serum from wethers during pretreatment periods at various times of the year. Open bars indicate hours of daylight. (**P < 0.01 v. April, June or August).](image-url)
Table 1. Levels of prolactin (ng/ml; means ± S.E.M.) in serum from groups of wethers (n = 4) before and during treatment with propionated steroids

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control weeks</th>
<th>Treatment weeks</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>±34-5</td>
<td>±43-8</td>
</tr>
<tr>
<td>Dihydrotestosterone propionate</td>
<td>147-7</td>
<td>124-3</td>
</tr>
<tr>
<td></td>
<td>±55-7</td>
<td>±42-2</td>
</tr>
<tr>
<td>Testosterone propionate</td>
<td>96-3</td>
<td>127-8</td>
</tr>
<tr>
<td></td>
<td>±27-9</td>
<td>±41-9</td>
</tr>
<tr>
<td>19-Hydroxytestosterone dipropionate</td>
<td>103-8</td>
<td>151-0</td>
</tr>
<tr>
<td></td>
<td>±14-2</td>
<td>±32-3</td>
</tr>
<tr>
<td>Oestradiol dipropionate</td>
<td>89-6</td>
<td>124-0</td>
</tr>
<tr>
<td></td>
<td>±35-6</td>
<td>±39-2</td>
</tr>
</tbody>
</table>

Described above. During the course of the experiment levels tended to decrease in the group treated with dihydrotestosterone propionate. In these animals and also in those receiving the oil vehicle the seasonal differences persisted throughout the treatment period. In the groups treated with 19-hydroxytestosterone dipropionate and oestradiol dipropionate levels increased over the 6 week period (Table 1) and seasonal differences tended to diminish. An analysis of variance was carried out on the data in Table 1 using the difference between means obtained both before and after treatment. The results which are summarized in Fig. 2, show that oestradiol dipropionate and 19-hydroxytestosterone dipropionate raised prolactin levels significantly whereas testosterone propionate and dihydrotestosterone propionate had no effect.

![Fig. 2](image.png)

**DISCUSSION**

Results from the pretreatment period indicate that prolactin concentrations in wethers vary with seasonal changes in daylength in a way that could have been predicted from experiments using artificial photoperiods (Pelletier, 1973; Forbes et al. 1975).
The rather high dosages of steroids used in this study were chosen for their ability to affect gonadotrophin levels (Parrott & Davies, 1979). In spite of this, the dose of testosterone propionate used did not alter prolactin levels or markedly affect seasonal variations. Larger doses (100 mg given every other day) apparently reduce prolactin concentrations in wethers exposed to 8 h of illumination, but not in those maintained on 16 h photoperiods (Pelletier, 1973). However, no differential response to testosterone propionate was found in the present experiment when the data from animals treated at various times of the year were examined. Moreover, as with other species (Shin et al. 1974; Dohler et al. 1978; Herbert, 1978), the results indicate a tendency towards increased, rather than decreased, levels during treatment with testosterone propionate. Similarly, the significant rise in prolactin concentration produced by oestradiol dipropionate parallels similar findings in the rat (Dohler et al. 1978).

The apparent decrease in prolactin levels during treatment with oil alone and with dihydrotestosterone propionate (Fig. 2) could have been due to adaptation of the animals to the blood collection procedure since stress is known to raise prolactin concentrations in ruminants (Bryant, Linzell & Greenwood, 1970). The lack of effect of dihydrotestosterone propionate, although it was administered at a dose sufficiently high to reduce gonadotrophin levels (Parrott & Davies, 1979), agrees with data recently obtained in the rat (Dohler et al. 1978). This suggests that the inability of dihydrotestosterone to undergo conversion to oestrogen may prevent it from influencing prolactin secretion and/or release. This conjecture is reinforced by the finding that 19-hydroxytestosterone dipropionate was more effective in raising prolactin levels than was testosterone propionate, but less effective than oestradiol dipropionate. 19-Hydroxytestosterone is an intermediate in the aromatization pathway from testosterone to oestradiol and it could thus be expected to be aromatized more readily than testosterone.

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REFERENCES


