Relationship between antler development and plasma androgen concentrations in adult roe deer 
(Capreolus capreolus)

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Summary. Four male deer (4–5 years old), living in normal park conditions, were captured every 2 weeks in the year to study annual variations in androgen levels and the relationship between testicular activity and the antler cycle. Data were also obtained from 77 wild adult males (>3 years old) caught in the forest. Plasma androgen concentrations rose briefly in April and markedly in August; the high values in August corresponded with the breeding period.

From the time of casting to maximum growth was about 60 days; mineralization of the antlers then lasted for 45 days only. The mineralization phase of antler growth corresponded to the reactivation of testicular function in late January (0.43 ± 0.16 to 1.99 ± 0.42 ng androgen/ml, N = 4).

Introduction

Much work has been done on the annual reproductive cycle in male deer. Seasonal changes in testicular and plasma testosterone have been observed in the roe buck, Capreolus capreolus (Short & Mann, 1966; Bramley, 1970; Barth, Gimenez, Hoffmann & Karg, 1976; Sempéré, 1978), the red deer, Cervus elaphus (Lincoln, Youngson & Short, 1970), the white-tailed deer, Odocoileus virginianus (McMillin, Keenlyne, Erickson & Jones, 1974) and the reindeer, Rangifer tarandus (Whitehead & McEwan, 1973; Leader-Williams, 1979). The annual antler cycle, which is under hormonal control, includes four stages: a first stage of rapid growth which can be compared to the germinal tissue of mammalian embryonal bones (Lojda, 1956, in Bubenik, Bubenik, Brown, Trenkle & Wilson, 1975b). At the end of this stage, the mineralization process interrupts the blood supply and the hard dead antlers develop. The third stage corresponds to the hard horn when velvet has disappeared. In the final stage, the old antlers are cast off after osteoclastic activity. There is a precise relationship between the antler cycle of roe deer and annual testosterone secretion (Barth et al., 1976) and the purpose of the present study was to analyse the annual cycle of plasma androgen concentrations in relation to the antler cycle in the captive roe deer and to relate the reactivation of genital function with the growth of antlers in captive and wild roe deer in winter.

Materials and Methods

Study area

This work was carried out in the Chizé Forest, located in mid-west France (46°10’N and 0°30’E) near the Atlantic coast. The climatic conditions are those of a temperate oceanic

0022-4251/81/030049-05$02.00/0
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climate with an average minimum temperature of 2°C in winter and an average maximum of 20°C in summer. The government-controlled forest is of 5000 ha, 2620 ha of which are entirely closed and constitute a National Reservation for the roe deer.

**Captive roe deer**

The 4 male deer were 4–5 years old in December 1978. These animals were used to living in a park of 2 ha and natural feeding was supplemented with oats and forage. Blood samples were collected every 2 weeks for 1 year. Sampling always began at 10:00 h and was always over by 12:00 h (noon). The animals were driven towards nets and restrained physically by 2 people for the sampling. A blood sample was withdrawn into heparinized tubes from the anterior femoral vein of each animal. After centrifugation for 15 min, the plasma samples were frozen until assay for androgen. The stages of development of the antlers were recorded as hard horn, shedding, growth, and full growth in velvet.

**Wild roe deer**

During November 1977–March 1978 and November 1978–March 1979, 77 adult bucks (>3 years old) were caught in the National Reservation of the Chizé Forest, and data were collected as described for the captive deer. Plasma androgen values obtained for 4 wild bucks shot whilst feeding normally in April (7.08 ± 0.66 ng/ml) were approximately the same as those obtained from animals in captivity in April (5.96 ± 0.68 ng/ml).

**Androgen assay**

Plasma concentrations were assayed in duplicate samples by a specific radioimmunoassay which did not involve chromatography. Plasma (0.5 ml) added to 0.1 ml [3H]testosterone (1000 c.p.m.) was extracted with 7 ml ethyl ether. The ether layer was transferred to a 10 x 75 mm test tube and evaporated at 37°C under a stream of nitrogen. Buffer (phosphate-buffered saline containing BSA), pH 4, was then added and the tubes vortexed to bring the androgen into solution. After addition of 0.5 ml of the testosterone antiserum (immunoabsorbant cellulosic-antitestosterone-3-(O-carboxymethyl)-oxime–bovine serum albumin) and 0.1 ml [3H]testosterone, the tubes were incubated at 4°C for at least 20 h, then centrifuged. Aliquots (0.5 ml) of the supernatant were placed into vials with 3 ml scintillation fluid and the amount of radioactivity was determined.

The specificity of the testosterone antiserum was checked against different steroids. Significant cross-reaction was obtained only with 5α-dihydrotestosterone (45%) and 5β-dihydrotestosterone (17.8%). Dihydrotestosterone was not separated from testosterone but in a separate study was found to represent 25.57 ± 2.45% (n = 10) of the total androgen concentration. Other steroids had a very low cross-reaction: 5β-androstane-3β,17β-diol (1.4%), androstanedione (1.4%). The sensitivity of the method was 70 pg androgen/ml plasma. The recovery was 88 ± 0.02%. The accuracy of the method was tested by assay of different volumes of plasma samples (0.2–0.7 ml); there was a good binding relationship in terms of volume between 0.4 and 0.7 ml plasma (y = 0.88 + 4.50 x: r = 0.9934). The interassay coefficient of variation was 7% (n = 6).

All data are presented as the mean ± s.e.m. Statistical comparisons were examined by analysis of variance and linear regression (Hewlett Packard 65 programs STAT 1-05A–STAT 1-22A).
Results

There were pronounced seasonal variations in the annual cycle of plasma androgens in captive bucks (Text-fig. 1). In autumn and early winter, hormone values were low. There was an increase of short duration in androgen concentrations in early February and this was also observed for captured wild bucks (Table 1). In the captive bucks, androgen values increased again in spring (March–April) and summer (July–August) (end of February–April, \( P < 0.05 \); June–July or August, \( P < 0.001 \)). The maximum values in July–August were followed by a rapid decrease at the end of August (early August–end of August, \( P < 0.001 \)).

Text-fig. 1. Annual variations (mean \( \pm \) s.e.m.) of plasma androgen concentrations in 4 captive roe bucks in relation to the antler cycle. B = breeding season.

<table>
<thead>
<tr>
<th>Date of capture</th>
<th>No. of bucks</th>
<th>Androgen (ng/ml)</th>
<th>Antler length (cm)</th>
<th>Date of capture</th>
<th>No. of bucks</th>
<th>Androgen (ng/ml)</th>
<th>Antler length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 Nov.</td>
<td>6</td>
<td>0.25 ± 0.05</td>
<td>1.25 ± 0.80</td>
<td>6 Nov.</td>
<td>6</td>
<td>0.40 ± 0.19</td>
<td>1.40 ± 0.87</td>
</tr>
<tr>
<td>6 Dec.</td>
<td>3</td>
<td>0.18 ± 0.03</td>
<td>6.80 ± 1.26*</td>
<td>5 Nov.</td>
<td>3</td>
<td>0.15 ± 0.01</td>
<td>8.83 ± 3.19*</td>
</tr>
<tr>
<td>13 Dec.</td>
<td>7</td>
<td>0.16 ± 0.03</td>
<td>7.80 ± 1.40</td>
<td>3 Nov.</td>
<td>3</td>
<td>0.15 ± 0.01</td>
<td>9.60 ± 0.57</td>
</tr>
<tr>
<td>20 Dec.</td>
<td>4</td>
<td>0.15 ± 0.04</td>
<td>14.70 ± 2.20*</td>
<td>2 Nov.</td>
<td>3</td>
<td>0.17 ± 0.03</td>
<td>11.60 ± 2.24</td>
</tr>
<tr>
<td>17 Jan.</td>
<td>6</td>
<td>0.15 ± 0.05</td>
<td>19.60 ± 1.28</td>
<td>1 Nov.</td>
<td>4</td>
<td>0.58 ± 0.08</td>
<td>18.50 ± 0.92*</td>
</tr>
<tr>
<td>24 Jan.</td>
<td>6</td>
<td>0.68 ± 0.05*</td>
<td>22.20 ± 1.04</td>
<td>30 Oct.</td>
<td>6</td>
<td>1.39 ± 0.19</td>
<td>19.60 ± 0.66</td>
</tr>
<tr>
<td>7 Feb.</td>
<td>5</td>
<td>0.67 ± 0.07</td>
<td>22.00 ± 1.50</td>
<td>29 Oct.</td>
<td>4</td>
<td>4.62 ± 1.22*</td>
<td>19.90 ± 1.43</td>
</tr>
<tr>
<td>14 Feb.</td>
<td>3</td>
<td>2.05 ± 0.50*</td>
<td>20.00 ± 1.06</td>
<td>31 Oct.</td>
<td>9</td>
<td>2.39 ± 1.28</td>
<td>21.30 ± 0.88</td>
</tr>
<tr>
<td>1 March</td>
<td>Velvet shedding period</td>
<td></td>
<td></td>
<td>Velvet shedding period</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Value significantly different from that preceding it, \( P < 0.05 \).

Antler growth begins in November, after casting. In wild buck, the antlers reach their full size in January after about 2 months of intense growth (Table 1). From November to January, antler growth was linear (\( y = 1.02 + 0.31x, r = 0.9941 \) in 1978–1979). Detailed study of the captive animals showed that the antler development period was 60 days and the antler mineralization period was 45 days (Table 2). Growth was again linear for the 60-day period of development (\( y = 3.50 + 0.22x, r = 0.9975 \)).
During the antler development period, the androgen concentration was very low but increased between Days 60 and 75 (Table 2).

A similar correspondence of the plasma androgen concentrations in relation to the antler cycle was observed in the wild animals (Table 1).

Table 2. Mean ± s.e.m. values for plasma androgen concentrations and antler growth in 4 captive roe bucks from the time of antler casting in November

<table>
<thead>
<tr>
<th>Days</th>
<th>Androgen (ng/ml)</th>
<th>Antler length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.12 ± 0.025</td>
<td>3.78 ± 0.26</td>
</tr>
<tr>
<td>15</td>
<td>0.27 ± 0.10</td>
<td>6.40 ± 0.29*</td>
</tr>
<tr>
<td>30</td>
<td>0.40 ± 0.23</td>
<td>10.15 ± 0.67*</td>
</tr>
<tr>
<td>45</td>
<td>0.57 ± 0.17</td>
<td>13.60 ± 0.34*</td>
</tr>
<tr>
<td>60</td>
<td>0.43 ± 0.16</td>
<td>16.00 ± 0.69*</td>
</tr>
<tr>
<td>75</td>
<td>1.99 ± 0.42*</td>
<td>16.60 ± 0.98</td>
</tr>
<tr>
<td>90</td>
<td>2.63 ± 1.59</td>
<td>17.20 ± 1.53</td>
</tr>
<tr>
<td>105</td>
<td>2.60 ± 1.07</td>
<td>17.20 ± 1.53</td>
</tr>
</tbody>
</table>

* Value significantly different from that preceding it, $P < 0.05$.

Discussion

The results obtained in this study are similar to those of others for testicular testosterone concentration (Short & Mann, 1966), or plasma testosterone in the roe deer (Barth et al., 1976). They confirm the existence of a long day-active testicular function in the roe deer. This species represents the only ungulate in the northern hemisphere which is a long-day breeder, but the relationship between the androgen levels and the antler cycle is similar to that for red deer (Lincoln et al., 1970) and white-tailed deer (Mirarchi, Scanlon, Kirkpatrick & Schreck, 1977) which are short-day breeders (Goss & Rosen, 1973; Mirarchi, Howland, Scanlon, Kirkpatrick & Sanford, 1978). In white-tailed deer and red deer, the time between the velvet cleaning and the rut is about 2 months, but in roe deer this span is almost 4 months (Bubenik, Bubenik, Brown & Wilson, 1977); the antlers of the roe buck grow during 2 months (November–December). As in all the cervids, this period is followed by the stage of mineralization correlated to the increase in plasma testosterone (Wislocki, Aub & Waldo, 1947; McMillin et al., 1974; Bubenik, Bubenik, Brown & Grota, 1974; Bubenik et al., 1977). In white-tailed deer, the injection of an antiandrogen (cyproterone acetate) blocks the process of mineralization (Bubenik & Bubenik, 1973; Bubenik, Bubenik, Brown & Wilson, 1975a) but does not affect velvet formation. The influence of the testosterone concentration differs according to the annual cycle of antler development. For animals in normal cyclic activity the decrease in androgen levels causes antler shedding. The start of antler growth may be attributable to the absence of testosterone (Blauel, 1935; Goss & Rosen, 1973). Testosterone treatment of castrated animals, with antlers in velvet caused casting of the velvet and, subsequently, of the antlers (Wislocki et al., 1947; McMillin et al., 1974). However, a low level of testosterone facilitates growth and probably starts the formation of the antler tissue (Thompson, Rodriguez, Kowarski, Migeon & Blizzard, 1972; Bubenik et al., 1975b). Other hormones interfere with the growth of antlers. Growth hormone (Bubenik et al., 1975b) and thyroxine play an important part in antler development (Lebedinsky, 1939), and gonadotrophin secretion is also correlated with the antler growth in the male deer (Markwald, Davis & Kainer, 1971).

Although the relationship between the cycle of the testicular activity and the antler growth...
cycle is not yet clearly defined in the cervids, it is important to establish that a phase relationship always exists between these two rhythms, even if the species breeding period occurs at different periods in the year. In the roe deer, antler growth is over in January when plasma androgen concentrations begin to increase and daylight increases. In all the other cervids, the greatest development of the antlers is reached after the summer solstice when plasma testosterone values are higher and daylight begins to decrease.

References


Received 12 June 1980