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General and Comparative Endocrinology 131 (2003) 21–31

GENERAL AND COMPARATIVE ENDOCRINOLOGY

www.elsevier.com/locate/ygcen

Effects of testosterone on pedicle formation and its transformation to antler in castrated male, freemartin and normal female red deer (*Cervus elaphus*)

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Accepted 4 November 2002

Abstract

Pedicles and antlers are male deer secondary sexual characters. As such, development of these structures is under the control of androgen hormones. Pedicle growth is caused by increasing and elevated plasma testosterone (T) levels, whereas first antler transformation from a fully formed pedicle occurs when the T levels are decreasing. Castration prior to pedicle initiation abrogates future pedicle and antler formation. Female deer also have the potential to develop pedicles and antlers, but they do not normally express this phenotype due to lack of sufficient androgen stimulation. Previous studies have shown that female white-tailed deer could be readily induced to grow pedicles as well as antlers by singular administration of exogenous androgens (EA), but in red deer (*Cervus elaphus*) singular or irregular EA treatment could only stimulate castrated male, normal or ovariectomised females to grow pedicles, but not antlers. The present study was set out to test whether these EA-induced pedicles in red deer failed to give rise to antlers was because they were constitutively incapable of doing so, or because the plasma T profile naturally exhibited in intact stags was not achieved by the androgen treatment used in these previous studies. Eight castrated red deer stag calves, 3 freemartins (females which were born co-twin to males), and 3 normal female red deer were used in the present study and treated with EA, either as biweekly injections for the castrates or as implants for freemartin and females until the late stage of pedicle growth. Blood sampling was carried out biweekly for the analyses of plasma T and IGF1 concentration. The results showed that the natural plasma T profile in the experimental deer was successfully mimicked through regular EA treatment and subsequent withdrawal at late pedicle growth stage. All castrated males, 2 out of 3 freemartin, and 1 out of 3 normal female red deer formed not only pedicles, but also antlers. Based on these results, we conclude that EA-induced pedicles at least in red deer of the *genus Cervus*, like those in the *genus Odocoileus*, are constitutively capable of giving rise to antlers, if they are of sufficient height.

Keywords: Antler; Pedicle; Deer; Testosterone; IGF1; Freemartin

1. Introduction

Antlers are deer cranial appendages, which are cast and regenerate each year from pedicles, permanent protuberances of the frontal bone. Deer start to develop pedicles when they approach puberty during their first year of life. First antler generation takes place spontaneously when the pedicles grow to the species-specific height, which is 5–6 cm in the red deer. The transformation from pedicles to antlers can be readily visualised as growing antlers, unlike pedicles which are covered with the typical scalp skin, are enveloped by a covering with short fine hair, known as velvet. In late summer prior to the rutting season, growing antlers become fully calcified and the velvet is shed to expose bare hard bone. Hard antlers are cast in the next spring and new antler regeneration follows immediately in the red deer. From then on, antler development enters a well-defined annual cycle (Fennessy and Suttie, 1985).

The potential to form a pedicle and an antler is exclusively held in the periosteum overlying the lateral crest of the deer frontal bone (Goss and Powel, 1985; Hartwig and Schrudde, 1974; Li et al., 2001a). Therefore, this periosteum is termed antlerogenic periosteum (AP). Histological examinations showed that the interior

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doi:10.1016/S0016-6480(02)00625-1

component (osseocartilaginous tissue) of the pedicle and antler is formed from AP (Li and Suttie, 1994), whereas the exterior component (velvet skin) is the derivative of scalp skin (Li and Suttie, 2000).

Pedicles and antlers are male secondary sexual characters. As such, pedicle formation and antler growth cycles are under the control of androgen hormones. Suttie et al. (1984 and 1991) reported that pedicle formation was caused by increasing and elevated plasma testosterone (T) levels, whereas first antler transformation from a pedicle took place when T levels were decreasing. Antler growth fell in the period when T levels were barely detectable. Antler calcification and velvet skin shedding were the consequence of high plasma levels of T, and antler casting and subsequent antler regeneration were associated with very low or undetectable levels of T (Bubenik, 1982; Suttie et al., 1991). Apart from androgen hormones, nutrition may also be involved in pedicle formation, because pedicle initiation tends to occur at a threshold body weight (about 56 kg in red deer), irrespective of age or season of the year (Suttie and Kay, 1982). Li et al. (1999) reported that cultured AP cells react to T by proliferating only in the presence of a sufficient levels of insulin-like growth factor 1 (IGF1). Therefore, nutritional cues may act synergistically with androgens to facilitate the development of pedicle through the growth factor pathway.

It is known that female deer also possess antler growth potential, but except for reindeer (*Rangifer tarandus*), where cows regularly grow antlers, females of other deer species do not normally express this phenotype due to lack of sufficient androgen stimulation (Goss, 1983). Females of non-reindeer species do occasionally grow antlers, but antlers grown by these females are abnormal in that they remain permanently in velvet (Chapman, 1975). The factors that cause female deer to grow antlers are not well understood, but Goss (1983) hypothesised that these antlered females could have been freemartins, that is, females which are born co-twin to males.

The first experimental induction of pedicle and antler growth was carried out by Wislocki et al. (1947). In their study, they successfully induced two ovariectomised white-tailed deer (*Odocoileus virginianus*) hinds to grow not only pedicles, but also antlers by administration of exogenous T. Along this line, Jaczewski (1981) and Jaczewski et al. (1976) treated normal or ovariectomised female red deer (*Cervus elaphus*) with T and induced pedicles, but not antlers, to grow. Likewise, Goss (1983) failed to induce antler formation by treating an ovariectomised female sika deer (*Cervus nippon*) with T, although the treatment caused pedicles to grow to 2–3 cm high. Prepubertally castrated red deer typically do not grow pedicles and antlers. T treatment to these castrated stags induced only pedicles but not antlers to grow (Jaczewski and Krzywinska, 1974). Why deer from genus *Odocoileus* can be readily induced to grow antlers, but deer from genus *Cervus* (red or sika deer) cannot, is not known. Jaczewski (1982) thought that this might be due to the specific differences in antler physiology of the species, but the underlying mechanism was not known.

To gain insights into the mechanism of antler generation from pedicles, recently we carried out two experiments using red deer (Li and Suttie, 2000; Li et al., 2001a). The results suggested that antler generation is the consequence of the interactions between the apical pedicle stem tissue and its overlying skin, and these two interactive tissues must become and remain intimately associated for the successful establishment of these interactions. Then, it follows that the reason antlers did not generate from the exogenous-androgen-induced (EAI) pedicles in the genus *Cervus* in previous studies may not be that these pedicles are constitutively incapable of doing so, but rather that the close association and interactions between the apical pedicle stem tissue and its overlying skin are not established and sufficiently maintained by the androgen hormone treatment used (singular or irregular administration). Indeed, Goss (1983) noticed that the apices of those pedicles induced by singular T administration underwent seasonal changes, they waxed and waned. On the other hand, that antlers could generate from the pedicles induced by a singular androgen administration in the genus *Odocoileus* could be because the tissue interactions are easier to establish than those in the genus *Cervus*. This claim is also supported by the fact that naturally antlered females occur most commonly in the genus *Odocoileus*, and much less commonly in the genus *Cervus* (Goss, 1983).

The present study was set out to test whether the plasma T profile naturally exhibited in intact male calves could be mimicked in castrated males, freemartins or female red deer by regular administration of exogenous T, followed by its withdrawal at late stage of pedicle formation. If so, this T profile could stimulate pedicle growth and effectively maintain the close association of the interactive tissues for an extended period, and hence promote spontaneous first antler generation from EAI pedicles.

2. Materials and methods

This study consisted of two experiments: Experiment I and Experiment II.

2.1. Animals

For Experiment I, eight castrated 6-month-old male red deer calves were used. For Experiment II, nine adult female red deer aged 4–6 years were available for study. Six of these were single born, normal females which had

a proven record of fertility. Three had been born co-twin to a male and were considered to be freemartins. Of these freemartins, two had been karyotyped as XX/XY chimeras; neither of them had ever calved despite having access to fertile stags and both had blind vaginas. The third was not karyotyped but had a blind vagina and had never calved. All the animals were kept at pasture under standard husbandry conditions throughout each study. Both Experiment I and II were approved by the local animal ethics committee and assigned as AEC Project No. 211 and AEC Project No. P99, respectively.

2.2. Treatment and sampling

2.2.1. Experiment I

The calves were castrated on 4 June (Southern hemisphere). The castration was conducted under xylazine sedation (1.8 ml/100 kg live weight i.v.; Rompun, Bayer NZ) and with local anaesthesia (Xylocaine, Asta Pharmaceuticals, NZ). Sedation was reversed with yohimbine (1 ml/40 kg live weight i.v.; Sigma Chemical, St. Louis, MO). After the removal of the testes, the scrotal wound was left open to drain.

Testosterone injection i.m. and blood sampling were carried out on the day of castration. Thereafter, injection and sampling were carried out simultaneously at biweekly intervals until the pedicles of each individual reached a height of 5 cm. Long acting testosterone enanthate (Schering) was injected i.m. in the neck at 250 mg/deer. A 10 ml sample of blood was withdrawn from jugular vein into a pre-heparinised tube and immediately centrifuged to collect plasma. The plasma was then frozen for later hormone assay. The second T treatment was conducted on 8 April of the next year to induce antler calcification and velvet shedding, and stopped on 13 May to trigger hard antler casting and new antler regeneration. Before casting, the hard antlers induced by the second T treatment were removed 1–3 cm above the junctions between pedicles and antlers to avoid injury.

2.2.2. Experiment II

On June 12, three of the normal females (allocated at random) and the three freemartins were implanted with six 30 cm pieces of silastic tubing (Dow Corning, MI), of outer diameter 0.46 cm and internal diameter 0.34 cm, packed with crystalline testosterone (T) (Sigma Chemical, St. Louis, MO). That the implantation approach was selected for this experiment is because the injection form of testosterone was not available at the time when this experiment was carried out. The implants were positioned in the groin region of each female under sedation and with local anaesthesia as described in Experiment I. Sedation was reversed with yohimbine. Care was taken to ensure implants were flat and lying parallel to one another. Half a year later (4 December), the implants were removed from the animals under anaesthesia as before. On June 5 of the following year, the three animals which had grown antlers were anaesthetised as before and eight 30 cm implants were positioned in the groin region. These implants were removed under anaesthesia on July 28. A 10 ml sample of blood was withdrawn into a pre-heparinised tube for hormonal analysis at two weekly intervals from 12 June until March 9 from all deer and from June 5 until November 13 from the three animals which grew antlers.

2.3. Measurement

The frontal bones were palpated for evidence of pedicles at each time when a blood sample was taken. The extent of pedicle/antler growth was measured with a wooden ruler (for pedicles) or a flexible plastic tape (for antlers). On several occasions the animals were weighed.

2.4. Testosterone radioimmunoassay

T was measured in plasma by radioimmunoassay after extraction for 5 min with 1 ml toluene:hexane (2:1 v/v) using the method of Garnier et al. (1978) with the following modifications. The antiserum used was raised in rabbits against T-3CMO BSA (Elder and Lewis, 1985) and the labelled ligand was [1,2,6,7-^{3}H]T (Amersham Product No. 1 TRK 402). The separation of free and bound T was carried out with Norit-A activated charcoal (J.T. Baker, Chemical, New Jersey). The sensitivity of the assay was 1 ng/ml ($n = 12$) and the inter- and intra-coefficients of variation (over 8 assays) for pools of stag plasma containing 2.74, 4.85, and 8.64 ng/ml were 10.27 and 17.20, 10.73 and 12.4, 10.55, and 18.63%, respectively. Deer plasma samples containing high levels of T were diluted in parallel to the standard curve of the assay. Recovery of tracer levels of labelled T was 62.08%.

2.5. IGF1 analysis

IGF1 was extracted from plasma using the method of Moore and Mylek (1993). Briefly, 100 μl of plasma were diluted in 900 μl of 20 mM HCl containing 0.02% Triton X-100. After 10 min, 800 μl of the diluted plasma were added to a small column containing 1.7 ml Sephacryl HR100 (Pharmacia LKB, Uppsala, Sweden). The liquid was run onto the column, and then the binding proteins were eluted with 800 μl of 20 mM HCl, 800 μl of 18 mM phosphate buffer pH 3.5 and 1000 μl of phosphosaline BSA (0.1%) buffer pH 7.5. The IGF1 was then eluted with 1600 μl of phosphosaline BSA (0.1%) buffer pH 7.5. The extracted IGF1 was measured using a double antibody (raised in sheep) radioimmunoassay. Intra-assay variation of plasma control pools was 14.7, 13.2, and 8.7%, whereas inter-assay variation was 19.1, 18.3, and

13.0% at 172, 407, and 807 ng/ml, respectively. Assay sensitivity averaged at 16 ng/ml.

2.6. Statistical methods

Data from repeated observations on each animal were summarised within experiments, and means (with standard errors of the mean (SEMs)) calculated on an animal basis. Differences in values between times were tested using a *t* test, and, where sufficient replication and homogeneity of variance pertained, a *t* test was used to compare control and non-control animals.

3. Results

Deer pedicle initiation and formation, antler initiation and formation in both experiments are shown in Figs. 1 (castrates) and 2 (freemartin and normal female). Mean pedicle and antler length, T, live weight, and IGF1 profiles for all sexes from both experiments are given in Figs. 3–5, and summary statistics for these profiles are given in Table 1.

3.1. Experiment I: castrated males

3.1.1. Pedicle and antler formation

Eight out of 8 castrated males formed both pedicles and antlers after T-treatment (Figs. 1 and 3; Table 1), although very small, but true antlers were formed on the left side of one stag (Fig. 1E; 3 cm in height) and on both sides of another stag (Fig. 1F; 1 cm in height). Of the 8 castrates, seven initiated their pedicles (Fig. 1A) 42–56 days after T-treatment commencement, while the remaining animal, which had the lightest initial bodyweight (52.5 kg compared to an average 54.9 kg) initiated pedicles at 84 days. Pedicles grew linearly for an average of 105 (range: 84–126) days (Figs. 1B and 3), at a rate of 0.40 (SEM 0.03) mm/d. The mean dates between initiation and termination of antler growth were 9 November (range: 8 October–16 December) and 19 February (range: 14 January–25 March), respectively. The mean antler size was 37.8 cm (range: 23–60 cm) (Fig. 1D). Antler growth rate ranged from 1.7 to 4.9 mm/d, with a mean of 3.1 (SEM 0.40) mm/d (Figs. 1C,D and 3). The second T-treatment effectively induced full antler calcification and velvet skin shedding (Fig. 1F and G). The cessation of the T-treatment triggered the hard antler buttons to cast and new antlers to regenerate (Fig. 1H).

3.1.2. Testosterone concentration

The exogenous T-treatment greatly elevated and maintained the plasma T levels in all the castrated deer (Fig. 4a). The mean T concentration from the first post-T-treatment sample until pedicle initiation was 3.85 (SED 0.43) ng/ml, which is significantly higher than 0.13 ng/ml for the pre-treatment sample ($P < 0.001$). All animals maintained high levels of T during pedicle growth, with an overall mean of 3.58 (SEM 0.24) ng/ml. The highest T value was detected on 13 August (Fig. 4A), which was associated with the mid-stage of pedicle growth. There was a general decline in T concentration toward the termination of pedicle formation and the initiation of antler growth, and a minimum mean across animals during this period was 1.77 (SEM 0.27) ng/ml. T-treatment concluded at the time of antler initiation and a mean of 0.73 (SEM 0.11) ng/ml was observed during antler growth. The mean T levels during the second T treatment period was 1.80 (SEM 0.30) ng/ml, an increase of 1.33 (SED 0.30) ng/ml from the previous sample.

3.1.3. Live weight

Live weight increased steadily throughout the experimental period (Fig. 5a), with pedicle initiation occurring at 54.9 (SEM 0.57) kg. There was a somewhat higher growth rate in spring–summer than winter (difference = 54 (SED 12.5) g/d; $P < 0.01$).

3.1.4. IGF1 concentration

There were highly significant changes in mean IGF1 concentration during the experiment period ($P < 0.001$; Fig. 5b). In particular, there was an increase of 173 (SED 16.7) ng/ml in IGF1 from the initial sample level to the winter plateau (first antler growth period). The small decline in mean IGF1 between 5 and 19 November aligns with a small drop in live weight at the same time. The mean maximum IGF1 concentration over all animals was 856 (SEM 56.6) ng/ml. The second T treatment to induce antler casting was associated with a significant elevation in IGF1 concentration, to an average of 444 ng/ml between 22 April and 20 May next year, from 252 (SED 12.6; $P < 0.001$) ng/ml on 8 April.

3.2. Experiment II: freemartins and normal females

3.2.1. Pedicle and antler formation

Two out of 3 freemartins (Fig. 2E) and one out of 3 T-treated normal females (Fig. 2F) formed both pedicles and antlers (Table 1), whereas the other freemartin and the 2 T-treated females only developed rudimentary pedicles reaching 2.0 cm in length, that did not give rise to antlers. All pedicle growth was initiated (Fig. 2A) 21–42 days after the T-treatment. The antler growth patterns of the two freemartins were very similar: antler growth initiated on 31 October and 20 November and reached maximum lengths of 40.5 and 38.0 cm on 26 February, respectively (Fig. 3). The antlers of the female started to grow on 4 December and attained an average length of 11 cm by 11 February (Fig. 3). No control females developed pedicles or antlers. The second

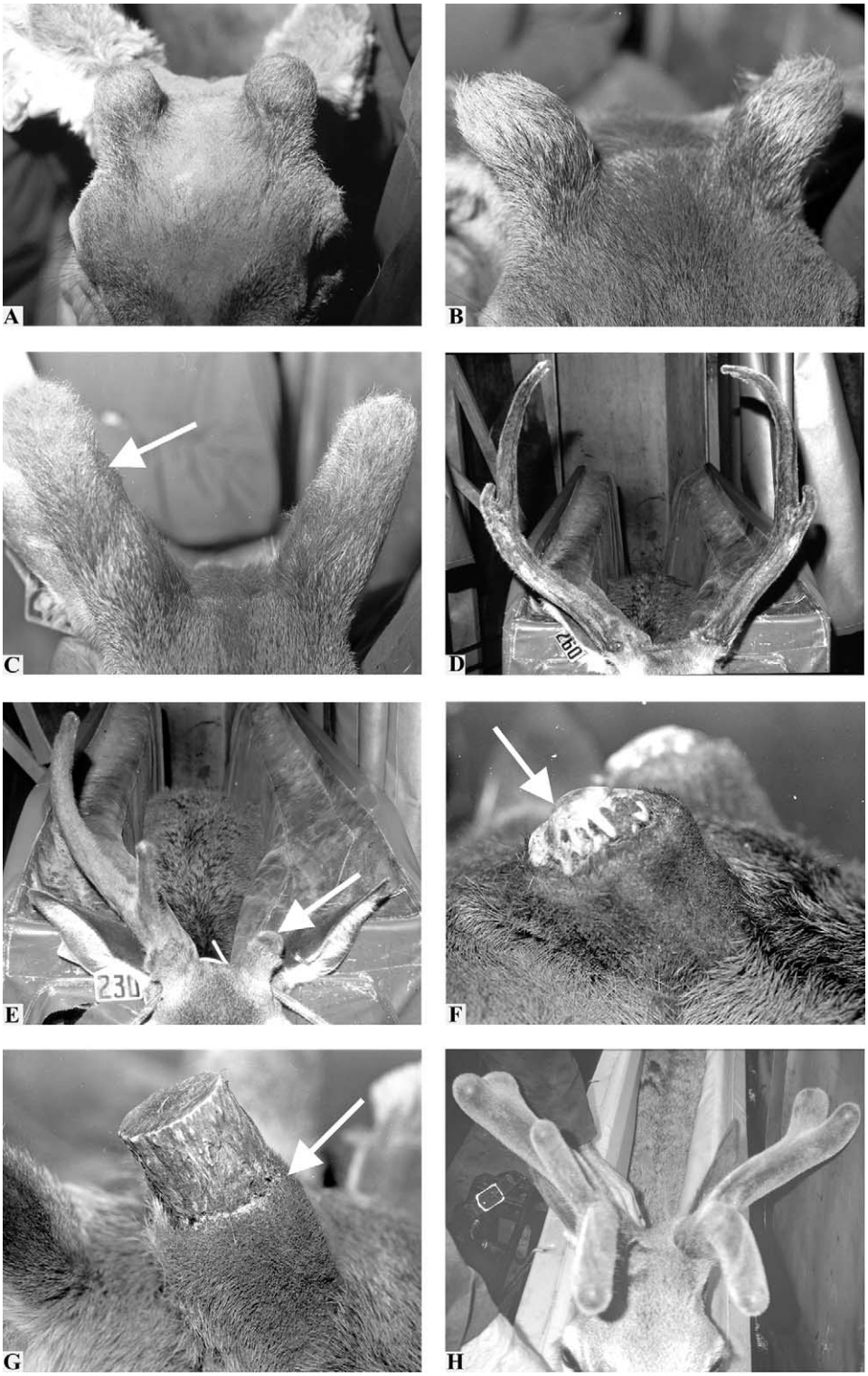

Fig. 1. Pedicles and antlers grown by prepubertally castrated male red deer calves after administration of exogenous testosterone. (A) Mid-growth stage pedicles. (B) Late-growth stage pedicles. (C) Fully formed pedicles with early growing antlers on their tops. Notice that the junction (arrow) between the pedicle and the antler can be visually identified by the difference in hair type. (D) Fully formed antlers just before velvet skin shedding. (E) Antlers formed by a stag. Notice that the difference in size between left-side (small antler bud, arrow) and right-side (fully formed antler). (F) Hard antlers (arrow) formed by a stag. Notice that although these antlers are small, they are true antlers as they became fully calcified after exogenous testosterone treatment. (G) Hard antler button. This button was generated following the removal of the antler followed by the second exogenous testosterone treatment. Notice the junction (arrow) between the living pedicle and the hard antler button. (H) Regenerated antlers following casting of the hard antlers/buttons.

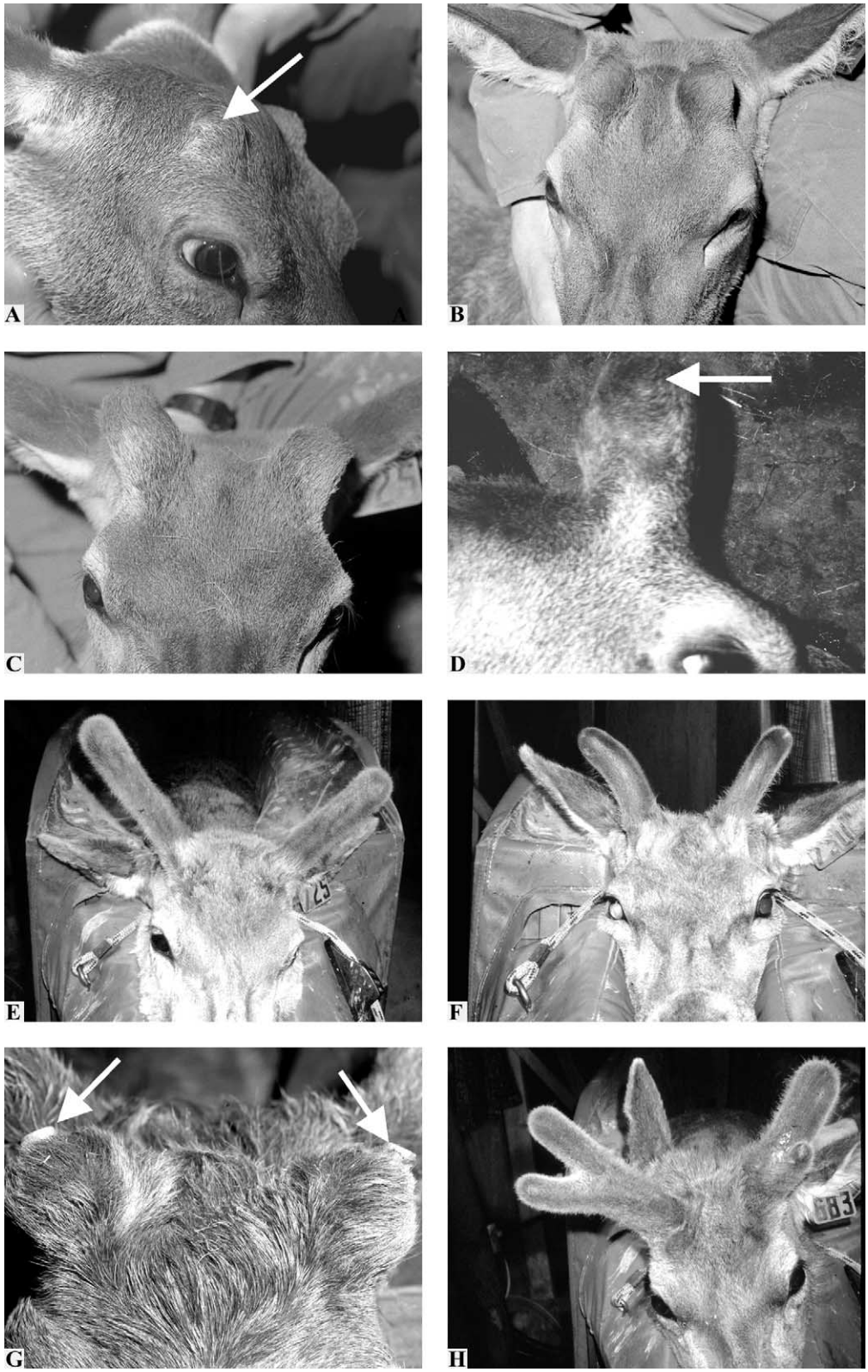

Fig. 2. Pedicles and antlers grown by freemartin or normal female red deer after the administration of exogenous testosterone. (A) An early-growth stage pedicle (arrow) grown by a freemartin. (B) Mid-growth stage pedicles grown by a freemartin. (C) Late-growth stage pedicles grown by a freemartin. (D) Transformation from a permanent pedicle to a deciduous antler in a freemartin. Notice the shiny velvet-like skin (arrow) on the top of the pedicle. (E) Mid-growth stage antlers grown by a freemartin. (F) Early-growth stage antlers grown by the normal female. (G) Hard antler buttons (arrows) on a freemartin. This button was generated following the same procedure as for Fig. 1G. Notice the junctions between the living pedicles and the dead antler buttons. (H) Regenerated antlers following casting of the hard antlers/buttons in a freemartin.

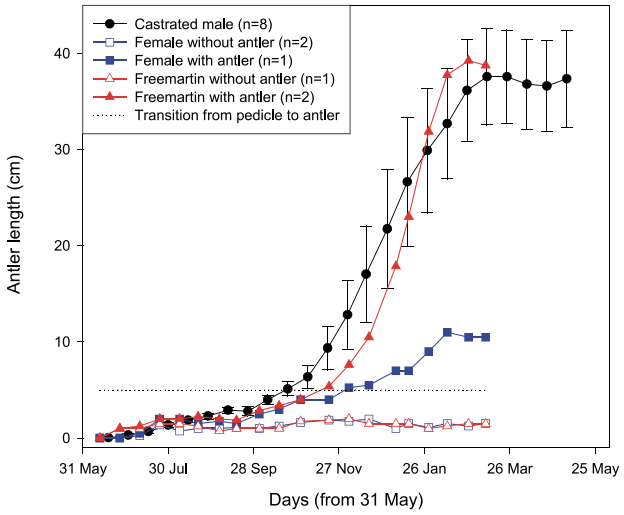

Fig. 3. Mean ± SED data for antler length in biweekly intervals during the period of the study from castrated stags ($n = 8$), freemartins ($n = 3$), testosterone treated females ($n = 3$).

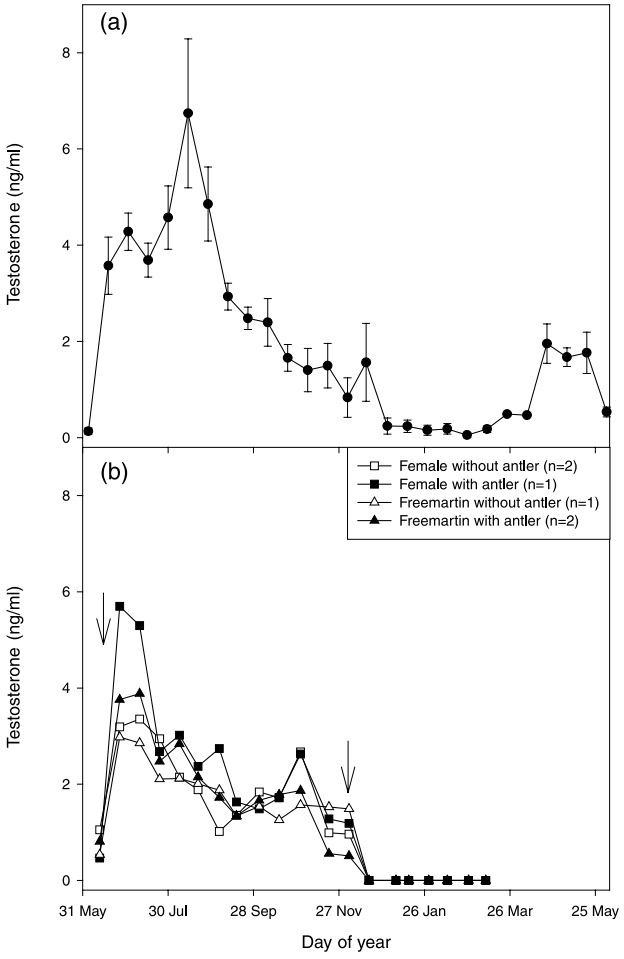

Fig. 4. Mean ± SED data for plasma testosterone concentration in biweekly intervals during the period of the study from (a) castrated stags ($n = 8$) and (b) freemartins ($n = 3$) and testosterone-treated females ($n = 3$). The two arrows indicate the time of testosterone treatment and withdrawal respectively.

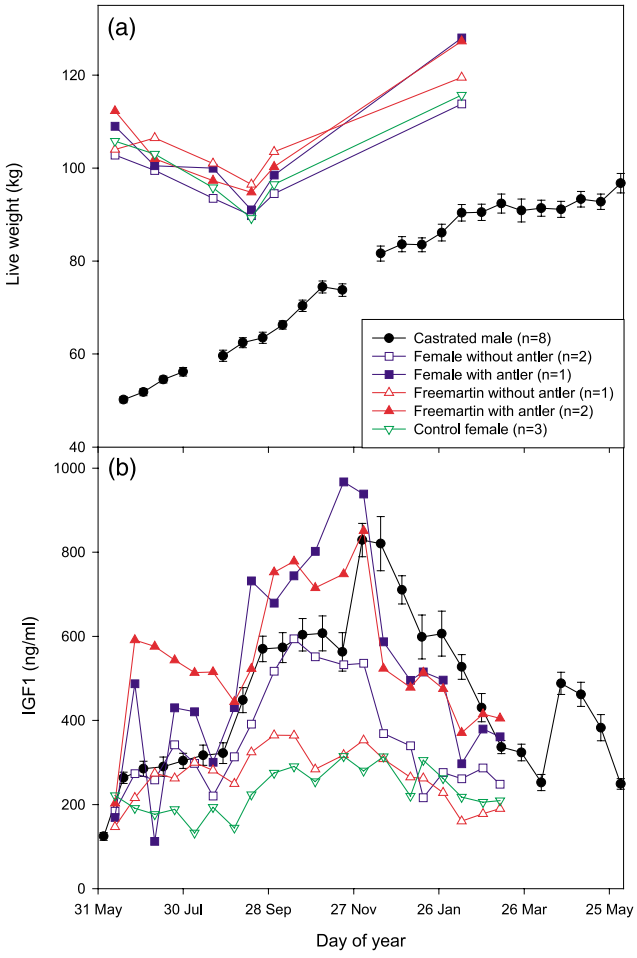

Fig. 5. Mean ± SED data for live weight (a) and IGF1 concentration (b) in biweekly intervals during the period of the study from castrated stags ($n = 8$), freemartins ($n = 3$), testosterone treated females ($n = 3$) and normal females ($n = 3$).

T-treatment induced the full antler calcification and velvet skin shedding (Fig. 2G). The cessation of the T-treatment triggered hard antler button casting and new antler regeneration (Fig. 2H).

3.2.2. *Testosterone concentration*

The exogenous T-treatment greatly elevated and maintained the plasma T levels in all the freemartin and normal female deer (Fig. 4a). The mean T concentration from the first post-T-treatment sample to pedicle initiation was 3.86 ng/ml, which was significantly higher ($P < 0.001$) than the mean for the pre-treatment sample of 0.79 (SED 0.58) ng/ml. The average T concentration during the period of pedicle growth until the withdrawal of the T treatment (4 December) was sustained around an average of 1.76 (SEM 0.07) ng/ml, a drop of 2.11 (SED 0.36) ng/ml from the pre-pedicle initiation period. No attempt was made to statistically contrast the differences in T profiles with sex or antler-growth status because of the minimal replication, but the T concentration was lower in the non-antlered freemartin and the

Table 1
Summary statistics from profiles for (a) pedicle and antler, (b) testosterone (T), (c) live weight and (d) IGF1 measurements presented in Figs. 3, 4, and classified by sex and antler status

Sex	Castrated male		Freemartin		Female + T		Control female	
Date of treatment (Southern hemisphere)	4 June		12 June		12 June		12 June	
Antler status	Antler		Antler	No antler	Antler	No antler	No Antler	
n	8		2	1	1	2	3	
	Mean	SEM					Mean	SEM
(a) Pedicle and antler								
Time of pedicle initiation (d)	51	5.3	21	28	42	42	—	—
Pedicle growth rate (mm/d)	0.40	0.03	0.28	0.07[a]	0.24	0.03[a]	—	—
Time of antler initiation (d)	156	10.4	151	—	175	—	—	—
Antler growth rate (mm/d)	3.1[b]	0.40[b]	3.2	—	0.8	—	—	—
Final antler length (cm)	37.8[b]	4.8[b]	39.3	—	11.0	—	—	—
Time of attaining final length (d)	260[b]	10.1[b]	259	—	244	—	—	—
(b) Testosterone (T)								
Initial T (ng/ml)	0.13[l]	0.07	0.81[l]	0.54[l]	0.47[l]	1.06	0.00	—
Before pedicle initiation (ng/ml)	3.98	0.42	3.89	2.92	4.56	3.17	0.00	—
During pedicle growth (ng/ml)	3.58	0.24	1.81[c]	1.69[c]	2.01[c]	1.62[c]	0.00[c]	—
During antler growth (ng/ml)	0.73[l]	0.11	0.00[d]	0.00[d]	0.00[d]	0.00[d]	0.00[d]	—
(c) Live weight								
Initial live weight (kg)	50.2	0.67	112.3	104.0	109.0	102.8	105.8	3.4
Live weight at pedicle initiation (kg)	54.9	0.57	105.0	105.5	100.0	97.5	—	—
Winter growth rate (g/d)[e]	136	11.3	−182	−78	−188	−135	−174	35.6
Spring–summer growth rate (g/d)[f]	190	10.1	220	155	250	162	179	5.2
(d) IGF1								
IGF1 on date of treatment (ng/ml)	124	9.3	203	147	169	184	221	35.0
Winter plateau[g] (ng/ml)	297	17.0	531	264	364	284	171	29.0
Spring transition[h] (ng/ml)	561	34.3	692	334	739	513	261	16.7
Summer peak[i] (ng/ml)	787	42.0	799	336	952	534	297	39.4
Late summer–autumn decline[j] (ng/ml)	598	52.3	463	234	462	292	254	34.8
Late autumn nadir[k] (ng/ml)	252	19.2	405	190	361	248	209	24.5

Times (d) are measured in days from date of treatment.
[a] Pedicle growth was measured to the cessation of T treatment on 4 December (175 d).
[b] $n = 7$. One animal completed pedicle development but only formed very small antlers; another animal grew one normal antler and one very small antler. These 3 small antlers were omitted for statistical analysis.
[c] Measured to the cessation of T treatment on 4 December.
[d] Measured subsequent to the cessation of T treatment on 4 December.
[e] For castrated males 4 June–24 September; for all other types of sex 12 June–16 September.
[f] For castrated males 24 September–11 February; for all other types of sex 16 September–11 February.
[g] For castrated males 18 June–27 August ($t = 6$); for all other types of sex 26 June–4 September ($t = 6$).
[h] For castrated males 10 September–19 November ($t = 6$); for all other types of sex 16 September–31 October ($t = 4$).
[i] For castrated males 3 December–31 December ($t = 6$); for all other types of sex 20 November–4 December ($t = 2$).
[j] For castrated males 14 January–25 March ($t = 6$); for all other types of sex 18 December–26 February ($t = 6$).
[k] For castrated males 8 April; for all other types of sex 9 March.
[l] Values below the sensitivity of the assay.

non-antlered females than in the antlered counterparts. T concentrations for the control females were below the minimum detectable level in all cases (data not shown).

3.2.3. Live weight

Live weight decreased during the winter period (31 May–16 September) at an average of 150 (SED 19.4) g/d, and increased over spring–summer (17 September–11 February) at a rate of 230 g/d for antlered animals compared to 170 (SED 12.5; $P < 0.01$) g/d for all non-antlered animals (including controls) (Fig. 5a).

3.2.4. IGF1

Mean IGF1 concentration for control animals decreased by 50 ng/ml from the pre-treatment sample level to the winter plateau (mid-stage of fast antler growth), whilst it increased by an average of 195 (SED 77) ng/ml ($P < 0.05$) for T-treated animals. Thereafter, control animals showed a moderate increase in IGF1 concentration to an average profile maximum of 348 (SEM 32.4) ng/ml, with a stronger response for freemartins and T-treated females over this period, leading to an average profile maximum of 723 (SEM 91.3) ng/ml. The IGF1

peak occurred slightly earlier for the female experiment than for the castrated male experiment (Fig. 5b).

4. Discussion

It is well accepted that, although androgen hormone treatment can readily induce pedicle growth in prepubertally castrated male deer or female deer (intact or ovariectomised), antlers can only generate from the exogenous-androgen-induced (EAI) pedicles in white-tailed deer (Wislocki et al., 1947), but not in red deer (Jaczewski, 1982) or sika deer (Goss, 1983). Therefore, it has been concluded that antler generation from EAI pedicles was deer species-dependent (Jaczewski, 1982). In sharp contrast to this currently held view, in the present study we successfully stimulated antler formation from the EAI pedicles in the castrated male (8 out of 8), freemartin (2 out of 3) and normal female (1 out of 3) red deer by solely using androgen hormone treatment and its subsequent withdrawal.

What had enabled us to succeed in stimulating antler formation from the EAI pedicles in the red deer in this study, while others had failed in previous ones, is the method of androgen hormone treatment. In contrast to the previous studies, in which singular (Goss, 1983) or irregular (Jaczewski and Krzywinska, 1974) androgen hormone treatment was employed, we administered testosterone (T) regularly (biweekly) or continuously (implantation) to the castrated male, or freemartin and female red deer until the time when antlers had visually transformed from the apices of the EAI pedicles. This experimental design was based on our hypothesis (Li and Suttie, 2000; Li et al., 2001a,b) that, as an organ, first antler generation must depend on the interactions between mesenchyme (M, apical pedicle stem tissue) and epithelium (E, epidermis of the overlying skin). To enable the establishment of these M–E interactions, the apical pedicle stem tissue and its overlying skin must become intimately associated. Further, an extended period of the close tissue association is required for the successful establishment of the E–M interactions in normal male red deer. This close tissue association is maintained by continuous pedicle growth, which is triggered and sustained by the plasma T profile exhibited in intact male calves. Therefore, mimicking this natural T profile will stimulate continuous pedicle growth, maintain the close association between the interactive tissues for a sufficiently long period, trigger the M–E interactions and eventually induce antler formation from EAI pedicles in red deer. In the present study, we have essentially achieved these expectations.

Androgen hormones control pedicle and antler growth through endocrine pathways (Suttie et al., 1995). By regular or continuous administration of T in the present study, we successfully achieved the natural plasma T profile in all our experimental deer. That is, the plasma T levels increased during the period of pedicle initiation, culminated at the early or mid stage of pedicle growth, and declined, thereafter, slowly and gradually toward the termination of pedicle growth and the initiation of antler formation (Suttie et al., 1991). Interestingly, the non-antlered freemartin and females had lower peak T levels than their antlered counterparts. Therefore, besides the T profile, the peak T level during pedicle growth may also play an important role in sustaining the late stage of pedicle growth and promoting antler generation.

The decline in plasma T levels toward the late stage of pedicle growth may be a prerequisite for triggering first antler initiation, as a high levels of T are detrimental to antler growth. This period occurs from August to December in both intact stag calves (Suttie et al., 1991), and the castrated, freemartin and normal female red deer in the present study. The decline in T levels in intact stags has been attributed to the decline in LH pulse frequency, which in turn impairs the secretion of T from the testes (Suttie et al., 1991). However, the decline in T levels in the present study cannot be attributed to the decrease of T production, as this T level decline took place just in the middle of exogenous T treatment. Consequently, this phenomenon can only be explained by increased clearance of the plasma T. Firstly, the T concentration may be diluted with the continuous body growth of the deer during this period. However, the results do not support this claim, as there was a three-month-overlap period when both body weight and T levels decreased simultaneously in the freemartin and the female deer. Secondly, clearance of T may be increased by binding to its target tissues. The only fast growing T target tissues common to the castrated male calves, the freemartins and the females are the pedicles (Li et al., 2001b). This claim is strongly supported by a recent finding that the T concentration in the growing pedicle tissue is 45 times higher than that in the growing antler tissue (unpublished data). This may be one of the reasons why the singular or irregular T administrations in previous studies (Goss, 1983; Jaczewski, 1982) could not sustain continuous pedicle growth in red or sika deer.

Because IGF1 levels are positively and significantly associated with first antler initiation (Suttie et al., 1989), we measured the plasma IGF1 concentration in the present study. The results showed that all the antlered deer and the two non-antlered female deer exhibited a natural plasma IGF1 profile. That is, IGF1 levels started to increase at the late stage of pedicle growth, reach the peak at the fast antler growth period, and then decrease gradually until the termination of antler growth (Suttie et al., 1989). What causes this IGF1 peak to happen is subject to speculation. The results from the present study demonstrated that this IGF1 peak is

closely associated with the previously elevated plasma T levels. Firstly, all the control female deer and the non-antlered freemartins which had lowest peak T level did not exhibit this natural IGF1 profile. Secondly, the non-antlered females, which had peak T level higher than the non-antlered freemartins but lower than the antlered deer, exhibited the normal seasonal IGF1 peak. Although, this IGF1 peak is lower than those of the antlered deer. Thirdly, the second period of T administration to stimulate the EAI antler to calcify, induced an increase in plasma IGF1 concentration. This finding is also supported by recently published results (Ditchkoff et al., 2001), in that IGF1 concentration is positively related to serum T levels. Consequently, the plasma IGF1 peak, which is associated with the late stage of pedicle growth, first antler initiation and formation, may be induced by the previously exhibited plasma T peak. The fact that antler growth was not induced in the two non-antlered females, despite the correct seasonal IGF1 peak, offers two possible explanations. Either this peak level was lower than the putative threshold, or the sensitivity to T stimulation of AP in females may be less than for castrates and freemartins, both of which had the exposure to T in prenatal life (Lincoln, 1973).

The results from the present study add further evidence to the findings that pedicle initiation relies on threshold deer body weight (56 kg for red deer, Suttie and Kay, 1982). It only took 21 and 42 days from the date of first T treatment for the freemartins and the females to initiate pedicle growth respectively, as the body weight of these deer at the time of T treatment was more than 100 kg, which is well over the threshold body weight. However, it took 51 days for the castrates to do so when these deer grew from average 50 kg (at the first T treatment) to 55 kg, near the threshold body weight. The present study also supports our previously advanced hypothesis that deer body weight plays a role in pedicle initiation through the growth factor pathway, particularly IGF1 (Li et al., 1999). Because, at the pedicle initiation period in the present study, body weight (108, 105, and 50 kg in freemartin, female and castrates respectively) was positively correlated with IGF1 levels (449.8, 371.3, and 301 ng/ml). Consequently, under the presence of sufficient T, pedicle initiation relies on threshold body weight, which may play the role through the IGF1 pathway.

Whether the first antler is initiated from EAI pedicles in red deer depends mainly on whether late stage of pedicle growth takes place. Nearly all the EAI pedicles in red deer (Jaczewski, 1982) or sika deer (Goss, 1983) in the previous studies only reached 1–3 cm high, and failed to give rise to antlers. It seems that antlers only grow when the induced pedicles exceed 5 cm in height. The results of the present study seem to support the notion that the peak level of IGF1 plays a pivotal role in sustaining late stage pedicle growth, because all the antlered deer exhibited the IGF1 peak, but both the control females and the non-antlered freemartin did not. Whether or not late stage of pedicle growth can proceed may depend on both the threshold IGF1 level and the sensitivity of antlerogenic periosteum to IGF1.

Wounding to the apical pedicle tissue has been found to be the most powerful way to stimulate antler growth from the EAI pedicles in deer of the genus *Cervus*, so long as the wounding includes both apical pedicle skin and its underlying stem tissue (Jaczewski et al., 1976; Jaczewski and Krzywinska, 1974). That wounding can rescue the failure of antler generation may be because wounding breaks the physical barriers (dermal tissue, subcutaneous loose connective tissue and fibrous layer of perichondrium) between the interactive tissues, which may facilitate these interactions and hence promote antler generation.

Based on the fact that eight out of eight castrates, two of three freemartins and one of three normal females developed first antlers from the EAI pedicles in red deer without pre-wounding in the present study, we conclude that EAI pedicles at least in red deer of the *genus Cervus*, like those in the *genus Odocoileus*, are constitutively capable of giving rise to antlers, if they are of sufficient height.

Acknowledgments

The authors wish to thank Mrs. Shirley Martin and Mr Rob Porteous for doing IGF1 and testosterone analyses.

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