Role of gonadal hormones in the regulation of the seasonal antler cycle in female reindeer, *Rangifer tarandus*

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The hormonal control of the seasonal antler cycle was investigated over 4 years in four adult female Norwegian reindeer, *Rangifer tarandus*, maintained in captivity at Tromsø, Norway (69°N). The antlers were fully redeveloped in August, cleaned of velvet in September, and were retained in hard antler throughout the winter before being cast in March or April. The cleaning of the antlers occurred at the seasonal onset of ovarian activity and was associated with an increase in the blood plasma concentrations of testosterone, androstenedione, oestradiol and progesterone. Ovariectomy in October resulted in premature casting of the hard antlers in that year. This was paralleled with a decrease in the plasma concentrations of oestradiol and progesterone, but not of testosterone and androstenedione. Antlers were redeveloped, cleaned of velvet and cast each year in the ovariectomized females, but the antler cycle was abnormal since the cleaning of the antlers began 1–3 months later than in intact females and was often incomplete, and the casting surface of the old antler was deformed. The hardening of the antlers in the ovariectomized animals in autumn was correlated with seasonal increases in the plasma concentrations of androstenedione, but not in the concentrations of other steroid hormones; provocation tests using GnRH and arginine vasopressin (AVP) illustrated that the adrenal gland was a likely source of androgens. The initiation of antler growth in spring always followed the seasonal increase in the secretion of prolactin. The overall conclusion is that a gonadal steroid hormone, possibly oestradiol, normally acts to induce and maintain the hard antler state in female reindeer. This hormonal mechanism acts to synchronize the antler cycle to the seasonal reproductive cycle with the hard antlers functioning as weapons in intrasexual competition in winter.

Introduction

In most species of deer only males develop antlers, and both the initial growth of the antlers at puberty and the seasonal replacement of the antlers in the adult are events dependent on the secretion of testosterone from the testes (Wislocki *et al.*, 1947; Chapman, 1975; Goss, 1983; Bubenik, 1990). This is evident since castration before puberty prevents the development of the antler pedicles from which the antlers grow, while the growth of the pedicles can be readily induced in castrated males, or in females, by treatment with testosterone. Furthermore, castration of adult males prevents the seasonal cycle in cleaning and casting of the antlers, and this sequence can be induced in castrated animals by administration and withdrawal of testosterone. In the normal seasonal cycle, the seasonal increase in the secretion of testosterone from the testes before the mating season induces the full maturation of the newly formed antlers, as well as inducing the sexual and aggressive behaviour associated with the rut, thus ensuring synchrony of

the reproductive events (Lincoln *et al.*, 1972). The maturation of the antler involves the shedding of the velvet-like skin to expose the underlying bone antler which dies to form the hard antler. This functions as an insensitive weapon used by males when competing for dominance and access to females in the rut (Lincoln, 1992). The hard antlers are retained several months after the rut as long as the circulating concentrations of testosterone remain high. The seasonal decline in testosterone permits the casting of the old antlers, a process which can be triggered at any time, when males are in hard antler, by castration (Goss, 1968). The antlers are regenerated in intact males during the non-mating season when the secretion of testosterone from the testes is lowest.

The reindeer/caribou (*Rangifer tarandus*) is the only species of deer in which both sexes normally produce antlers (Leader-Williams, 1979). In contrast to other species of cervids, the antlers begin to develop well before puberty, and removal of the gonads of either sex soon after birth does not prevent the growth and subsequent seasonal replacement of the antlers (Skjenneberg and Slagsvold, 1968; Wika, 1980; Lincoln and Tyler, 1991). Nevertheless, it appears that testosterone plays an

Fig. 1. Summary of the seasonal antler cycle and other reproductive events in Norwegian reindeer, *Rangifer tarandus*.

important role in the regulation of the seasonal antler cycle in reindeer, at least in males. In reindeer bulls, the cleaning of the velvet to form the hard antlers occurs during the seasonal increase in the blood concentrations of testosterone before the rut (Whitehead and McEwan, 1973; Leader-Williams, 1979) and administration of testosterone to animals in velvet induces premature cleaning of the antlers (Ryg, 1983). In addition, casting of the antlers in early winter occurs following the seasonal decline in testosterone secretion after the rut (Whitehead and West, 1977; Leader-Williams, 1979; Mossing and Damber, 1981), and castration of adult reindeer in hard antler causes early casting of the antlers (Skjenneberg and Slagsvold, 1968). In long-term castrated animals the velvet is not cleaned rapidly in the early autumn, as it is in the intact animals, and the antlers are retained throughout the winter instead of being cast in early winter. The conclusion based on these observations is that in male reindeer, the secretion of testosterone by the testes, although not essential for the growth and seasonal replacement of antlers, has an overriding influence on the timing of the antler cycle, as in other species of cervid.

The hormonal regulation of the seasonal antler cycle in female reindeer has not been investigated in detail. The circulating concentrations of testosterone are low throughout the year in females, and it is therefore unlikely that testosterone regulates the antler cycle (Leader-Williams, 1979). In females, cleaning of the antlers normally occurs close to the onset of ovarian activity in the autumn and the hard antlers are retained throughout the winter (Fig. 1). Pregnant animals normally cast their antlers at about the time of calving in spring, while non-pregnant individuals cast a few weeks earlier (Espmark, 1971; Gagnon and Barrette, 1992). These observations indicate that the increased secretion of ovarian hormones during the autumn and winter may induce cleaning of the velvet and the development of hard antlers. The ovarian hormone oestradiol

is a possible candidate to mediate this effect, since it has been shown that the administration of oestradiol in other species of deer (sika and red deer) acts like testosterone to induce the hard antler state (Goss, 1968; Fletcher, 1978). Such a role of ovarian hormones would ensure synchrony between antler development and the annual reproductive cycle; in females, the hard antlers are used as weapons to exert social dominance especially when competing over feeding sites in the snow in winter (Henshaw, 1969).

The aim of the current study was to measure the seasonal changes in the plasma concentrations of a range of sex steroid hormones in female reindeer to assess their possible involvement in the control of the seasonal antler cycle. Observations were made before and after ovariectomy with the prediction that if ovarian hormones act to induce and maintain the hard antler state during the winter, ovariectomy at this time should cause premature casting of the antlers. Since the adrenal glands may secrete sex steroids which could affect the antler cycle, provocation tests were performed to assess possible sources of the circulating steroid hormones. The seasonal changes in the plasma concentration of prolactin were measured in all animals, as prolactin may be one of the factors stimulating the initiation of antler growth in spring (Mirarchi *et al.*, 1978).

An abstract describing the acute effect of ovariectomy on the antler cycle in female reindeer has been published (Tyler and Lincoln, 1991).

Materials and Methods

Animals

Observations were made on four adult female Norwegian semi-domesticated reindeer from April 1989 to May 1993. The animals were maintained outdoors in adjacent enclosures at the Department of Arctic Biology, University of Tromsø, Norway (69°N, 19°E), and were given a concentrated pelleted diet, RF-71 (Jacobsen and Skjenneberg, 1979) or RF-80, with water or snow *ad libitum*.

The reindeer were pregnant at the start of the study and each produced a calf in May 1989 which remained with its mother throughout the period of observations. All four females were ovariectomized in October 1989. This was carried out under sterile conditions while the animals were deeply sedated using a single i.m. injection of 80–100 µg medetomidine kg⁻¹ body weight (Farnos Group, Turku) (Tyler *et al.*, 1990). Lidocain (xylocain, 10 mg ml⁻¹; Astra, Sodertalje) was administered locally around the ovarian pedicle and each ovarian pedicle was ligated using two absorbable sutures passed through the midline before the ovaries were removed. Sedation was reversed using a single i.m. injection of 400–500 µg atipamezole kg⁻¹ body weight (Farnos) (Tyler *et al.*, 1990), and all animals made an uneventful recovery. The ovaries were fixed in 10% formalin and later sectioned to record the presence of follicles (< 1.0 mm diameter) and corpora lutea.

Collection of blood samples and antler measurements

Heparinized blood samples (10 ml) were collected from the jugular vein of each animal approximately every week throughout the study. The plasma was separated within 60 min

and stored at -20°C until the concentrations of hormones were measured by radioimmunoassay. On three occasions before ovariectomy (May, July and October 1989) and on four occasions after ovariectomy (May, July, October and December 1990), blood samples (5 ml) were collected every 10 min for 4 h during a standard set of provocation tests. These involved the i.v. injection of GnRH ($1.0\text{ }\mu\text{g}$ in 2 ml saline; Ayerst Laboratories Ltd, Andover, Hants) after the first blood sample to stimulate the pituitary–gonadal axis, and the i.v. injection of arginine vasopressin (AVP $20\text{ }\mu\text{g}$ in 2 ml saline; Sigma Chemical Co., Poole, Dorset) to stimulate the pituitary–adrenal axis 2 h later. This latter provocation test was based on the observations that AVP stimulates a dose-dependent increase in the secretion of adrenocorticotrophin hormone (ACTH) in sheep (Ssewanyana *et al.*, 1990) and ACTH stimulates the release of adrenal androgens. The dose of GnRH was selected on the basis of the amount required to induce a physiological increase in the plasma concentration of LH in sexually active male reindeer (G. A. Lincoln, unpublished results) and the dose of AVP was based on the amount required to induce a physiological increase in the plasma concentration of ACTH in sheep (Ssewanyana *et al.*, 1990). The injection of GnRH was thought not to affect the response to AVP given 2 h later, since the two hormones act through different receptors in different tissues. To permit the collection of blood samples at frequent intervals without disturbance, the animals were retained in a pen and the samples were taken from a cannula inserted into the jugular vein at least 7 h before each study. The cannula terminated in a three-way tap and was kept patent with heparinized saline ($10\,000\text{ i.u. sodium heparin l}^{-1}\text{ }0.9\%$ (w/v) NaCl).

For each animal, the state of the antlers was recorded every week, and during the growing phase in 1992 (third year of the study) the length of each antler was measured approximately every month. Cast antlers were weighed and the volume determined by immersion in water. For comparison with the experimental animals, information on the dates of casting and cleaning of the antlers in adult female and male reindeer living in the locality was obtained from records kept at the Department of Arctic Biology and at the Reindeer Research Station, Lodingen.

Radioimmunoassays

The concentrations of testosterone and androstenedione in all the blood plasma samples were measured by radioimmunoassay, using the method for testosterone as described by Corker and Davidson (1978) and modified for an iodinated tracer (Sharpe and Bartlett, 1985), and for androstenedione as described by Baird *et al.* (1976). Both assays involved extraction of 0.4 ml plasma with diethyl ether. The sensitivity (90% B:Bo) of the testosterone assay was 8.0 pg ml^{-1} plasma and the intra- and interassay coefficients of variation (CV) were 11.9% and 16.4%, respectively, based on 14 assays. The corresponding values for the androstenedione assay were 7.0 pg ml^{-1} , 9.7% and 13.6%.

Progesterone concentrations were measured in the weekly plasma samples using a routine radioimmunoassay (McNeilly and Fraser, 1987). This assay had a minimum detection limit of

78 pg ml^{-1} plasma and intra- and interassay CVs of 7.7% and 10.1%, respectively. Oestradiol concentrations were measured by R. Webb (Edinburgh) in a small number of weekly samples collected before and after ovariectomy using a radioimmunoassay (Webb *et al.*, 1985). The sensitivity of the assay was approximately 0.5 pg ml^{-1} based on the extraction of 3.0 ml plasma. Prolactin concentrations were measured in all the weekly plasma samples using the method of McNeilly and Andrews (1974) which has previously been validated for the assay of prolactin in plasma from red deer (Curlewis *et al.*, 1988). This assay had a sensitivity of $0.8\text{ ng NIH-P-S15 ml}^{-1}$ plasma, and the intra- and interassay CVs were 10.9 and 5.9%, respectively, based on the mean of low, medium and high quality control samples measured in three assays.

Statistical analysis

The changes in the plasma concentrations of testosterone, androstenedione, progesterone and oestradiol at three stages of the antler cycle in the intact female reindeer and at one stage following ovariectomy were analysed using analysis of variance (ANOVA), and the pair-wise comparisons were assessed for significance by Newman–Keul's test. The mean dates for casting and cleaning the antlers were calculated using days numbered from 1–365 (1 January–31 December) and re-converting back to the date after determining mean $\pm$ SEM (Table 1). When data for more than one year were combined, the mean was determined for each animal and these were used to calculate the group mean $\pm$ SEM. Comparisons were made using Student's *t* test and the Mann–Whitney *U* test.

Results

Antler cycles

The seasonal changes in the antlers before and after ovariectomy are summarised in Fig. 2 and Table 1. At the start of the study, the experimental females were in hard antler. The antlers were cast from 19 April–25 May, which was close to the time of calving (16–28 May) and new antlers regenerated in the summer during the period of lactation. Cleaning of the velvet began in mid-September and the animals were again in hard antler by October. In non-pregnant females living in adjacent enclosures, the date of casting of the antlers was about a month earlier than that observed in the pregnant group, but cleaning occurred at a similar time in autumn (Table 1).

Ovariectomy in October caused the hard antlers to be cast in winter, 4–7 months earlier than in intact females (three animals cast 17–25 days and the fourth cast 77–96 days after ovariectomy). After casting, there was a delay before regrowth of the antlers commenced in March (Fig. 2). New antlers developed during the summer and these were fully grown in July (Fig. 3a). Shedding of the velvet began in October–December, 1–3 months later than in intact females, but was completed only in one animal (R13 Fig. 2). In the other three, the cleaning was protracted and was not completed during the winter (Fig. 3b). Parts of the antlers became broken off and the consequent bleeding from the damaged bone indicated that some of the antler tissue was still living.

Table 1. Summary of seasonal antler cycle in intact and ovariectomized female reindeer, *Rangifer tarandus*, kept outside at the Department of Arctic Biology, Tromsø, Norway

Antler cycle	Intact ^a pregnant/lactating	Intact ^b non-pregnant	Ovariectomized ^c
Date of casting (days)	14 May ± 3.5	8 April* ± 6.8	1 April* ± 14.7
Date of cleaning (days)	20 September ± 4.1	15 September ± 3.1	23 October*† ± 6.7
Duration of cleaning (days)	9.5 ± 2.2	11.3 ± 3.3	111.4*† ± 4.9

Values are means ± SEM. *n* = 4 animals per group.

^aExperimental group: 1989 data only; ^bnon-experimental group living in adjacent enclosures: 1990, 1991 and 1992 data combined;

^cexperimental group after ovariectomy: 1990, 1991 and 1992 data combined.

*Significantly different from Intact^a ($P < 0.05$ Student's *t* test); †significantly different from Intact^b ($P < 0.05$ Student's *t* test).

Fig. 2. Seasonal antler cycles over 4 years in (a) one control and (b) four experimental adult female reindeer living at the Department of Arctic Biology, Tromsø, Norway. The experimental animals were ovariectomized (OVX) in October 1989. Stages of the antler cycle are shown as periods of growth (velvet, □), cleaning (▨) and hard antler (■) terminating with casting of the old antlers (vertical lines). The special events are also shown: *1, antler broken; *2, part antler cast.

In the following spring, the remaining parts of the antlers were cast in three animals and new antlers developed, in some cases before the old ones were cast (Fig. 3c). In the fourth animal (R6 Fig. 2), regrowth of antler tissue included part of the old antler. The regrowth was complete by July but again the shedding of the velvet was delayed and protracted. In three animals, the antlers were cast during the winter or early spring having become cleaned of velvet a few weeks earlier. A similar seasonal cycle in the antlers of the ovariectomized animals occurred in the following year. Regrowth of the antlers began in April, the new antlers were completed by July and the shedding of the velvet began in October with considerable variation between individuals (Fig. 2).

The characteristics of the antlers developed by the ovariectomized reindeer are summarized in Table 2. The antlers of the ovariectomized females were similar in length, number of points and density to those developed by the intact, non-pregnant females but were less robustly built and weighed less. The casting surfaces of the antlers of the ovariectomized reindeer were different from those of intact females. In the latter, the antler was usually eroded at a point approximately 5 mm below a pronounced antler burr and the casting surface of the antler was flat or slightly convex. In the ovariectomized reindeer, the antlers were eroded at the level of the burr which was irregular or even absent and the casting surface was either flat or distinctly concave.

Fig. 3. Photographs of the experimental female reindeer living at the Department of Arctic Biology, Tromsø, Norway: (a) ovariectomized female with fully developed antlers in velvet in September 1990; (b) ovariectomized female in November 1990 showing partly cleaned antlers, and (c) ovariectomized female in April 1991 showing growth of a new antler around the base of an old antler that has not yet been cast.

Hormone cycles

The weekly changes in the plasma concentrations of the sex steroid hormones and prolactin in a representative female reindeer are illustrated in Fig. 4, and the results for all four animals are summarized in Figs 5 and 6. During May and June of the first year (before ovariectomy), when the animals were in the early phase of regrowing their antlers, the plasma concen-

trations of testosterone, androstenedione, progesterone and oestradiol were low, while the concentrations of prolactin were close to the seasonal maximum. By October, when the growth was complete and the velvet was shed from the antlers, the circulating concentrations of all four steroid hormones had increased significantly (Fig. 5), while the concentrations of prolactin had declined.

Ovariectomy in October resulted in a significant decrease in the plasma concentrations of progesterone and oestradiol, but not of testosterone and androstenedione (hard antler versus cast, Fig. 5). Inspection of the ovaries removed from the females revealed the presence of a single large corpus luteum (diameter 9.5–10.1 mm) in the ovaries of each animal along with multiple (11–33) follicles > 1.0 mm in diameter.

In the long-term ovariectomized females, the plasma concentrations of androstenedione, but not testosterone, varied significantly ($P < 0.01$, ANOVA) with time of year. The concentrations of androstenedione were highest during the autumn and winter when the antlers were in the protracted velvet shedding phase and lowest in mid-summer when the antlers were redeveloping (Fig. 6). The plasma concentrations of progesterone and oestradiol were below the detection limit of the assay at all times. There was a clearly defined seasonal cycle in the plasma concentrations of prolactin in the ovariectomized females with maximum values in summer and minimal values in winter (Fig. 6).

Hormone provocation tests

The i.v. injection of GnRH (1 µg per animal) caused no consistent change in the plasma concentrations of testosterone and androstenedione in the female reindeer, but the i.v. injection of AVP caused an increase in the plasma concentrations of testosterone and androstenedione on all occasions, with concentrations at a maximum 20–30 min after the injection (Fig. 7). The concentration of testosterone relative to androstenedione at the peak of the response was approximately 1:4. The magnitude of the responses increased significantly after ovariectomy (mean maximum testosterone: 38.2 ± 0.5 versus 106.5 ± 12.6 pg ml⁻¹ plasma; mean maximum androstenedione: 161.6 ± 11.4 versus 280.8 ± 8.4 pg ml⁻¹ plasma, before and after ovariectomy, respectively, $P < 0.01$), with a relatively larger increase in the testosterone response. There was no

Table 2. Summary of antler characteristics in intact and ovariectomized female reindeer, *Rangifer tarandus*, kept outside at the Department of Arctic Biology, Tromsø, Norway

Antler characteristics	Intact ^a pregnant/lactating (n)	Intact ^b non-pregnant (n)	Ovariectomized ^c (n)
Length (cm)	—	63.3 ± 3.9 (4)	59.5 ± 2.5 (8)
Points (number)	2.7 ± 0.4 (7)	3.6 ± 0.8 (5)	3.6 ± 0.3 (17)
Weight (g)	137.1 ± 18.8 (7) [†]	307.4 ± 45.8 (5) [*]	178.6 ± 14.5 (18) ^{†*}
Density (g cm ⁻³)	1.44 ± 0.01 (4)	1.47 ± 0.13 (5)	1.45 ± 0.72 (14)

Values are means $\pm$ SEM; n = 4–18 animals per group.

^aExperimental group: 1989 data only; ^bnon-experimental group living in adjacent enclosures: 1992 data only; ^cexperimental group after ovariectomy: 1990, 1991 and 1992 data combined except for length (1992 only).

^{*}Significantly different from Intact^a ($P < 0.01$ Mann–Whitney U test); [†]significantly different from Intact^b ($P < 0.01$ Mann–Whitney U test).

Fig. 4. Seasonal changes in the blood plasma concentrations of testosterone, androstenedione, progesterone and prolactin before and after ovariectomy (OVX) in an adult female reindeer. Stages of the antler cycle are shown as periods of growth (velvet, □), cleaning (▨) and hard antler (□) terminating with casting of the old antlers (vertical line).

Fig. 5. Summary of the changes in the blood plasma concentrations of testosterone, androstenedione, progesterone and oestradiol at various stages of the antler cycle before and after ovariectomy (OVX) in four adult female reindeer. Values represent means ($\pm$ SEM, $n = 4$) based on blood samples collected weekly for 6 weeks during each stage. * ($P < 0.05$); ** ($P < 0.01$).

seasonal change in the effects of AVP during the period while the animals were intact (provocation tests: May, July and October, Fig. 7) or after ovariectomy (provocation tests: May, July, September and December, Fig. 7).

Discussion

The results provide clear support for the hypothesis that in female reindeer the ovaries secrete a hormone that induces and maintains the hard antler state throughout autumn and winter. This was best demonstrated by the acute effect of removal of the ovaries in October from females in hard antler. Ovariectomy at this time resulted in the premature casting of the hard

antlers in early winter many months earlier than normal. This effect had never been observed previously following the same type of handling and drug immobilization; thus we conclude that functionally active ovaries normally act to maintain the attachment of the hard antlers in autumn and winter. In addition, the long-term observations of the ovariectomized females revealed abnormalities in the seasonal cleaning and casting of the antlers. In particular, the shedding of the velvet was delayed and protracted; in some animals parts of the antlers were cast at unusual times during the winter soon after cleaning; and sometimes regrowth commenced in spring without the complete loss of the old antlers. From these observations, we conclude that in the normal female, it is the increase in the secretion of sex steroids from the ovaries in the

Fig. 6. Summary of the seasonal changes in the blood plasma concentrations of testosterone, androstenedione and prolactin in four adult ovariectomized female reindeer. Values represent the half-monthly mean ($\pm$ SEM, $n = 4$) based on blood samples collected weekly over two consecutive years. Horizontal bars indicate range in times of casting and onset of cleaning of antlers.

autumn that induces the rapid and complete maturation of the antler to produce the functional hard antler.

The effects of ovariectomy on the antler cycle in females are essentially similar to the effects of castration in males. Castration of male reindeer in hard antler results in casting of the antlers after about a month, while long-term castrated males continue to cast and regrow their antlers annually, although the cleaning of the velvet in autumn is delayed and often incomplete (Skjenneberg and Slagsvold, 1968; Wika, 1980). Since testosterone induces the maturation of the antler and shedding of the velvet in reindeer, as in other species (Ryg, 1983), it is logical to conclude that the seasonal increase in the secretion of testosterone from the testes

before the mating season induces the shedding of the velvet in the male and that the hard antlers are retained because of the continued secretion of testosterone throughout the period of increased testicular activity.

In female reindeer, it is unlikely that testosterone is the principal gonadal hormone regulating the antler cycle. The peripheral blood concentrations of testosterone are very low in females compared with those in males ($< 1/250$ during the sexual active period of the seasonal cycle, G. A. Lincoln and N. J. C. Tyler, unpublished data). Furthermore, the blood concentrations of testosterone did not decline significantly after the removal of the ovaries in October, which triggered the casting of the antlers. The possibility that the adrenal gland is the main source of androgens in the peripheral circulation in females is supported by the results of the provocation tests which showed that AVP, and not GnRH, stimulated a marked increase in the plasma concentrations of testosterone and androstenedione at all times of the year. AVP is likely to act by inducing the release of ACTH from the anterior pituitary gland (Asher *et al.*, 1989; Ssewanyana *et al.*, 1990).

The ovarian influence on the antler cycle in female reindeer probably involves the secretion of oestrogens. In the study reported here, the shedding of velvet from the antlers in the intact females was associated with an increase in the peripheral blood concentrations of oestradiol. This coincided with the onset of ovarian cyclicity as judged by the increase in plasma concentrations of progesterone in late September, and the presence of a single large corpus luteum in the ovaries of each animal in late October. Furthermore, removal of the ovaries resulted in a decrease in the plasma concentrations of oestradiol and casting of the hard antlers. Oestradiol is known to be more potent than even testosterone at inducing mineralization of the antler and shedding of the velvet, or maintaining the hard antler state when administered to male deer of other species (Goss, 1968; Fletcher, 1978; Morris and Bubenik, 1982). Thus, it is most likely that oestradiol (or a metabolite) plays the principal role in inducing and maintaining the hard antler condition in female reindeer.

The timing of the period in hard antler in relation to the period of pregnancy in reindeer is also consistent with this effect of oestradiol on the antler. The hard antlers are retained throughout pregnancy during winter, and are normally cast about the time of calving in spring. The pattern of oestradiol secretion during pregnancy has not been documented in reindeer; however, in other ruminants the circulating concentration increases progressively especially in the second half of pregnancy, and declines abruptly at parturition (white-tailed deer: Harder and Woolf, 1976; Plotka *et al.*, 1977; domesticated sheep and goat: Challis, 1971; Challis and Linzell, 1971). It is a common observation that pregnant female reindeer retain their antlers longer than do non-pregnant animals (Espmark, 1971; Leader-Williams, 1988; Gagnon and Barrette, 1992). Lent (1965) was the first to suggest that it might be a seasonal decline in oestrogens that triggers antler casting in female caribou. Progesterone could be invoked as a regulator of the antler cycle related to pregnancy, but there is no experimental evidence to indicate that progesterone affects the tissue of the developing antler.

The female reindeer in the study reported here continued to cast and regrow antlers annually, and similar results have been

Fig. 7. Short-term changes in the blood plasma concentrations of testosterone and androstenedione following i.v. injection of GnRH (1 μ g) or arginine vasopressin (AVP) (20 μ g) at three times before (May, July and October), and four times after (May, July, September and December) ovariectomy (OVX, broken line) in four adult female reindeer. Values represent means $\pm$ SEM. Stages of the antler cycle are shown as periods of growth (velvet, $\square$), cleaning ($\square$) and hard antler ($\square$) terminating with casting of the old antlers (vertical line).

reported for a single ovariectomized adult reindeer (Tandler and Grosz, 1913). The persistence of antler cycles in castrated male reindeer is also well documented (Skjenneberg and Slagsvold, 1968; Wika, 1980). This cyclicity in the absence of any influence from the gonads may indicate that the seasonal changes in the secretion of adrenal androgens also influence the antler. This contention is consistent with the observation that there was a seasonal cycle in the peripheral blood concentrations of androstenedione in long-term ovariectomized females which correlated with the antler cycle. It is possible that other adrenal androgens such as dehydroepiandrosterone might be secreted in parallel with androstenedione to cause the protracted maturation of the antler in the gonadectomized reindeer in autumn and winter. Alternatively, the androgens may be metabolized to oestrogens within the antler. The observation that the adrenal androgen response to AVP increased after ovariectomy is also relevant and may indicate that the adrenal gland has the potential to secrete more adrenal androgens after removal of the gonads owing to some form of adrenal hypertrophy. It has been suggested that the differences in adrenal androgen secretion between species explain the differences in the type of antler produced by a male after castration (for example unregulated growth to form a 'perruque' versus relatively normal growth (Bubenik *et al.*, 1987; Bubenik, 1990)). The reindeer may represent the more extreme situation of a species adapted to a highly seasonal climate where the adrenal androgen secretion is sufficient in the autumn or winter to cause almost normal mineralization of antler and death of the velvet. This would result in death of the antler tissue and permit casting of the antler once the androgen concentrations decline in spring.

Casting of the hard antlers occurs when osteoclasts migrate to the junction between the living pedicle and the dead tissue of the hard antler, triggered by the changing concentrations of sex steroid hormones (Goss *et al.*, 1992). These cells erode the bone matrix and cause the casting of the old antler. In some species, new antler growth starts at the time of casting, while in others there is a delay before regrowth begins. A delay of several months occurs in adult male reindeer in which casting of the hard antlers normally occurs in November or December soon after the rut, but new antler growth does not begin until April. This also occurred in our female reindeer following ovariectomy in October. In this situation, the females cast their antlers prematurely in the early winter but regrowth did not begin until March. The dissociation between casting and regrowth has led to the concept that regrowth of the antlers is stimulated by a separate hormonal mechanism from that regulating casting (Waldo and Wislocki, 1951). Prolactin has been proposed as a trophic hormone that might provide a stimulus to antler growth in spring, on the basis of the observation that blood concentrations of prolactin increase in spring in white-tailed deer coincident with regrowth of the antlers (Mirarchi *et al.*, 1978). However, it has been shown that the suppression of prolactin secretion in spring and early summer using bromocriptine does not affect the rate of antler growth (Bubenik *et al.*, 1985). Experimental studies in several mammals have demonstrated that prolactin is involved in controlling the growth and moulting of the pelage in spring (Lincoln, 1989). The increase in the secretion of prolactin at this time appears to stimulate reactivation of mitotic activity in the hair follicles, and induce the development of the hair type characteristic of the summer pelage. Since the spring moult and

regrowth of the pelage commences at the same time as regrowth of the antlers in most temperate climate species of deer including reindeer, it is possible that prolactin is involved in the reinitiation of antler growth in spring.

In conclusion, the study reported here provides the first clear experimental evidence that the seasonal cycle in gonadal activity regulates the seasonal cycle in cleaning and casting of the antlers in female reindeer. The increase in secretion of oestrogens by the ovaries in autumn and winter may induce the development of the hard antlers which are then retained throughout the period of ovarian activity. In the absence of the gonads, the seasonal changes in the secretion of adrenal androgens appear to be sufficient to induce partial maturation of the antler in winter and regulate the antler cycle, a feature unique to reindeer. The hormonal regulation of the antler cycle in the normal situation ensures that the antlers are fully developed as functional weapons when competition is most intense. In females, this is related to competition over food in the winter, while in males, this is related to competition for mates in the autumn.

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