Zoological Studies

Testosterone Levels in Male Formosan Reeve's Muntjac: Uncoupling of the Reproductive and Antler Cycles

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Kurtis Jai-Chyi Pei, Kerry Foresman, Bing-Tsan Liu, Long-Hwa Hong, and John Yuh-Lin Yu (2009) Testosterone levels in male Formosan Reeve's muntjac: uncoupling of the reproductive and antler cycles. *Zoological Studies* **48**(1): 120-124. Cervid species are generally assumed to be seasonal in their reproductive activity, and species that develop antlers also do so in a seasonal manner in conjunction with this annual puberty. As gonadotropin endocrine support for reproductive activity wanes, the resultant lowered testosterone levels initiate antler casting. Male Formosan Reeve's muntjac (*Muntiacus reeves micrurus*) exhibits an annular antler cycle with growth initiating in early May, velvet shedding and antler hardening by early Sept., and casting the following May. This cycle was correlated with fluctuating testosterone levels in a manner somewhat similar to that observed in other cervids. However, this species remains reproductively active year-round with spermatozoa present in the testes and epididymides, with no variation in their quantity or quality. These findings suggest that the testosterone threshold required for antler development in this muntjac may be set higher than that required for spermatogenesis, or conversely, that spermatogenesis might be controlled by other hormones in addition to testosterone. http://zoolstud.sinica.edu.tw/Journals/48.1/120.pdf

Key words: Antler cycle, Formosan Reeve's muntjac, Fecal testosterone, *Muntiacus reevesi*, Seasonal levels.

The annual cycle of male cervids has traditionally been divided according to their reproductive state into the phases of reproductive activity and quiescence. The shift between these 2 phases involves changes in the appearance and size of the testes and accessory glands, as well as endocrine secretion and spermatogenesis (see a review by Lincoln 1985). Because of their resemblance to puberty in other mammalian species, the phenomenon of annual recrudescence of the cervid male reproductive system is called "annual puberty" (Lincoln 1971a b). In most male cervids, annual fluctuations in secretions of certain gonadotrophic and gonadal hormones dictate the development of some secondary sexual characteristics, such as a long neck mane, an enlarged neck (Lincoln 1971a), and especially the re-growth of antlers (Bubenik 1982, Lincoln 1985, Suttie and Simpson 1985, Bubenik and Bubenik 1987).

Male deer cast off their antlers when, after a period of intensive mating activity, their testosterone levels rapidly decrease, below certain threshold levels. A new set of antlers (covered by velvet) begin to grow almost immediately in some species such as red deer (*Cervus elaphus*),

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sika deer (*C. nippon*), wapiti (*C. canadensis*), roe deer (*Capreolus capreolus*), and fallow deer (*Dama dama*); but in others, such as white-tailed deer (*Odocoileus virginianus*), reindeer (*Rangifer tarandus*), and moose (*Alces alces*), new antler growth may begin only after a period lasting from several weeks to several months (Bartos 1990). The growth of velvet antlers stops about 3-4 mo later, before the beginning of another rutting season, and soft antlers begin to mineralize into hard antlers in response to rising levels of testosterone. The velvet stage in the antler cycle is therefore associated with the quiescent phase of the reproductive cycle. Additional experiments also show that both female and castrated male deer can be induced to grow pedicles by high testosterone or androgen levels (Wislocki et al. 1947, Lincoln 1985), which suggests that females also have the potential to grow antlers; only their regular testosterone level is too low to trigger the antler-growing mechanism (Bubenik 1982).

Unlike other deer species which have been studied, indirect evidence indicates that male muntjacs (genus *Muntiacus*), despite demonstrating an annual antler cycle, do not exhibit "annual puberty" and remain fertile throughout the year. Intensive sexual activity has been observed in captive Reeves' muntjac (*M. reevesi*; Barrette 1975, Pei and Liu 1994, Liu and Liu 2004) and wild Indian muntjac (*M. muntjak*; Barrette 1977) in the velvet stage of the antlers. Births in wild populations of Reeves' muntjac occurred evenly throughout the year in England based on a study of 153 road-killed individuals collected from 8 different locations between 1963 and 1983 (Chapman et al. 1984); similarly, another field study in Taiwan found that although there were birth peaks at 7-8 mo intervals, Reeves' muntjacs were clearly un-seasonal breeders (Pei et al. 1995).

Chapman and Harris (1991) documented that there were no seasonal changes in the size and activity of testes, epididymides, or accessory reproductive glands in wild Reeves' muntjac in England. However, despite castration causing earlier hard antler casting and a cessation of mineralization of velvet antlers, as in temperate-zone deer species, the same study found only minor, but statistically non-significant, seasonal changes in serum testosterone levels (Chapman and Harris 1991). The authors further suggested that the antler cycle of male muntjacs might be controlled by other hormone(s) in addition to testosterone. Liu et al. (2004) used an artificial

vagina to collect semen from 10 live animals monthly, and found that although no differences in semen volume or sperm motility score occurred among the 4 seasons, sperm concentrations did significantly decrease during the summer months (early May to early Aug.) compared to the other seasons.

Given that male muntjacs display an annual, synchronized antler cycle but appear to remain fertile throughout the year, we examined the correlation between testosterone levels and these physiological states. Our goal was to determine whether or not testosterone levels indeed are not well-defined, as in other deer species studied, on a yearly basis (Chapman and Harris 1991), and how this hormone might be involved in synchronization of the antler cycle. Fecal testosterone levels in tame, captive adult male Formosan Reeve's muntjacs were measured during both cycles.

MATERIALS AND METHODS

Fresh fecal samples from 4 individually housed adult male muntjacs were collected bi-weekly from 15 Nov. 1997 to 15 Jan. 1999 at the Experimental Deer Farm of the Department of Animal Science, National Pingtung University of Science and Technology, Pingtung, Taiwan. The antler status was checked every day, and the initial dates of hard antler casting, velvet shedding, and shedding completion were recorded for each individual. Fecal samples were stored at -25°C until being analyzed, and then were oven-dried at 60 ± 2°C for 24-48 h to a constant mass. Dry fecal samples were mixed completely with $CHCl_3$ and NaOH, and all steroids were extracted from the top layer using diethyl ether (Yang 1990). Fecal testosterone levels (ng/g dry matter) were determined by a radioimmunoassay using the techniques of Yu et al. (1988). The antiserum was produced in rabbits by immunization with testosterone-3-carboxymethyloxime and bovine serum albumin (Steraloids, Newport, RI, USA). Tritium-labeled steroids were incubated with aliquots of steroids from the samples dissolved in 0.01 M phosphate-buffered saline (PBS) and 0.1% gelatin for competitive binding with the antibody. Dextran-coated charcoal (0.5%) was employed to separate bound steroids from free ones. Supernatants containing the bound, labeled steroids were analyzed in a liquid scintillation counter.

Fecal testosterone levels were grouped into

23 consecutive 15 d periods throughout the entire antler cycle. Each fecal sample was assigned to a 15 d period based on the number of days it was collected before or after the starting date for each phase, namely casting, velvet shedding, and hard antler phases. The mean testosterone level of all samples for each period was used to represent the fecal testosterone level for the middle day of that period.

RESULTS

The 4 adult male muntjacs observed during the present study exhibited a clearly synchronized annual antler cycle. Hard antlers were cast, on average, on 2 May (± 15.8 d); velvet antlers began to grow a couple of days later and lasted for an average of 80.2 ± 17.3 d. The velvet shedding began by 27 July (± 2.8 d) and was completed by 9 Sept. (± 14.5 d).

In total, 112 fresh fecal samples were collected and analyzed for testosterone content (ng/mg dry matter). The percent cross reactions of the antiserum were tested for testosterone (100%), 5α-dihydrotestosterone (0.4%), and androstenediol (0.6%). The detection limit was 5 pg/assay tube. Since a chromatographic separation procedure of steroids following diethyl ether extraction was omitted, the concentration of androgen in

the sample was expressed as a testosterone equivalent, extrapolated from the standard curve. The intra-assay coefficient of variation was 6.5% (n = 8), and the inter-assay coefficient of variation was 10.1% (n = 8).

The fecal testosterone level on the day when hard antler casting occurred was low at 7.42 ± 3.62 ng/gm dry matter (n = 8), and it remained at low levels (6.79 ± 3.26 ng/gm dry matter; n = 23) throughout the velvet antler growing period. The fecal testosterone content increased dramatically to 20.38 ± 9.22 ng/gm dry mater (n = 15) when velvet was being shed from the antler. This level was then maintained (20.59 ± 9.29 ng/gm dry matter; n = 66) throughout the entire hard antler phase before casting occurred. The equation: $Y = 0.0001X^5 + 0.0099X^4 - 0.2654X^3 + 3.1948X^2 - 14.8320X + 27.7730$, where Y is the fecal testosterone level and X is the 15 d period throughout the antler cycle, was found to be the best-fitting multiple-equation (R^2 = 0.70) to describe seasonal changes in fecal testosterone levels in male muntjacs (Fig. 1).

DISCUSSION

The timing of antler replacement observed was identical to that reported for muntjacs in both wild and captive populations over a broad
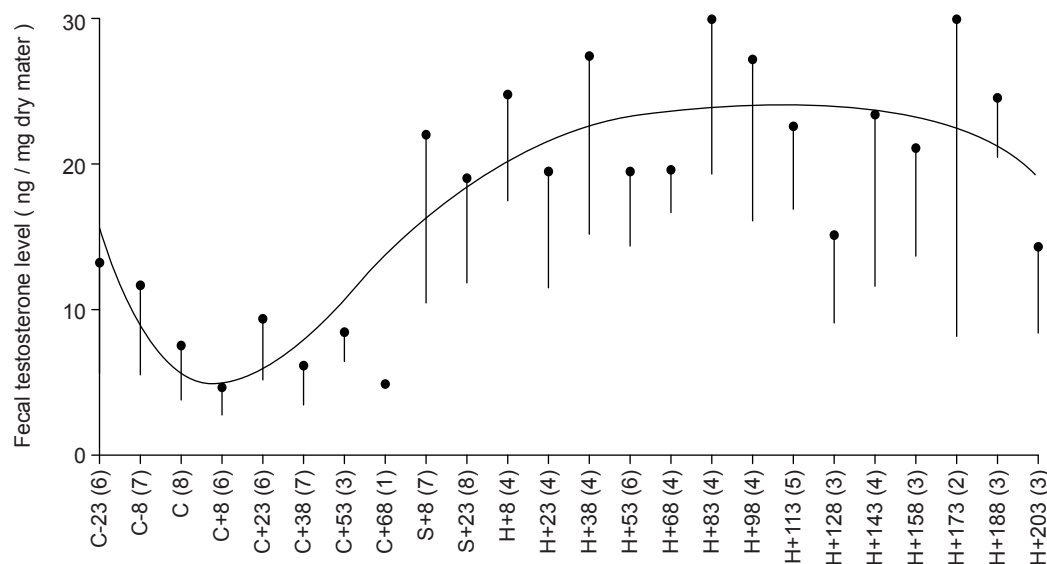
Fig. 1. Changes in fecal testosterone levels (mean ± 1 S.D.) during different phases of the antler cycle of the male muntjac (*Muntiacus reevesi micrurus*). C indicates the date of hard antler casting, S indicates the velvet shedding phase, while H indicates the hard antler phase. A minus or plus sign indicates the number of days before or after the phase which is indicated began. Numbers in parentheses are the sample size.

geological area (see Pei 1990 for review). We also found that the testosterone level of adult male muntjacs fluctuated throughout the year in correlation with their annual antler replacement cycle (Fig. 1), a pattern which is very similar to other deer species studied and clearly shows that the quantity of circulating testicular androgens in muntjacs also plays a key role in regulating phase shifts in their antler cycle, as suggested earlier by Pei and Liu (1994). The less-well-defined seasonal changes in testosterone levels found by Chapman and Harris (1991) may have been due to the effects of stress, since individuals examined in their study were obtained by hunting. The prevalent control of testicular androgen production by photoperiod through the hypothalamus-pituitary-gonad axis which is well established in other deer species (Bubenik 1990) may, therefore, also apply to muntjacs.

The surprisingly synchronous antler cycle in muntjacs over broad regions (Pei 1990) is unique among subtropical and tropical species. Most deer species in the subtropics and tropics, although experiencing individual annual rutting periods, show very weak or no synchronization in their antler cycle or reproductive activity, even within a local population (Goss 1963 1969, Schaller 1967, Lincoln 1985, Jackson and Langguth 1987). Presumably, such asynchronization results from a lack of selection for birth to occur during a certain period of the year. When seasonally cyclic events do simultaneously occur among individuals in a tropical or subtropical population, they are most likely triggered by some local phenomenon, such as rainfall patterns, vegetation growth patterns, diurnal temperature differences, etc., rather than photoperiod (Schaller 1967, Goss 1969, Branan and Marchinton 1987). These external regulators function not only locally, but also seem to be species-specific. Therefore, different populations of the same species or different species in the same location are usually not synchronous (Schaller 1967, Blouch 1987, Mishra and Wemmer 1987, Redford 1987).

Recent studies showed that another subtropical cervid species, the pampas deer (*Ozotoceros bezoarticus*), might also exhibit a similar phenomenon. The pampas deer has an annual antler cycle (Tomás 1995), but exhibits a year-round, but moderately seasonal, reproductive pattern (Ungerfeld et al. 2008). However, unlike the muntjac, their fecal testosterone concentrations were significantly correlated with both the reproductive behavior and antler phases (Pereira et al. 2005), which is more similar to other deer species (Ungerfeld et al. 2008).

Interestingly, although the low testosterone levels from early May (when casting occurred) to early Aug. (when velvet shedding began) coincided with the period of significantly low sperm concentrations found in an earlier semen collection study (Liu et al. 2004), the ability to produce fertile spermatozoa in male muntjacs apparently, unlike deer of temperate regions, does not completely cease during the velvet growing stage. This may be due to the following reasons. The threshold level of circulating testosterone required for initiating hard antler casting is higher than that required for spermatogenesis in the muntjac. Thus the annual fluctuation of testosterone levels in male muntjacs will never drop lower than that needed for maintaining basic sperm production, while the level in spring-summer will be low enough to trigger the casting of hard antlers. Another possibility is that spermatogenesis in muntjacs is only partially controlled by testosterone levels, and there may be other hormones involved as well. Other hormones, such as follicle-stimulating hormone or prolactin, which are also important for spermatogenesis (Bubenik 1990), might not vary seasonally. Prolactin, although not the prime factor in initiating gonadal reactivation in sheep in autumn (Lincoln et al. 2001), has been shown to be involved in regulating seasonal testicular activity and spermatogenesis in this species (Bubenik 1990, Lincoln et al. 1996, Jabbour and Lincoln 1999). Thus Formosan Reeve's muntjac appears to exhibit a unique uncoupling of the antler cycle from the reproductive pattern, which deserves further investigation.

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