

## Seasonal changes in plasma testosterone levels in the male South African hedgehog (*Atelerix frontalis*)

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Testosterone levels in plasma collected at monthly intervals from 6 to 15 male South African hedgehogs (*Atelerix frontalis*) housed in captivity showed a marked seasonal pattern. Values were relatively low ( $< 0,25$  ng/ml) from February to June but increased significantly from July to August. Testosterone levels peaked in August ( $\bar{x} = 2,85 \pm 1,37$  ng/ml;  $n = 15$ ) and December ( $\bar{x} = 2,73 \pm 0,36$  ng/ml;  $n = 6$ ) remaining relatively high from August to January. This seasonal pattern is similar to that recorded in the European hedgehog (*Erinaceus europaeus*).

Testosteronvlakke in plasma wat maandeliks versamel is van 6 tot 15 Suid-Afrikaanse krimparkies (*Atelerix frontalis*) wat in gevangenskap aangehou is, toon 'n seisoenale patroon. Waardes was laag ( $< 0,25$  ng/ml) vanaf Februarie tot Junie maar het vanaf Julie tot Augustus betekenisvol toegeneem. Testosteronvlakke het 'n piek bereik in Augustus ( $\bar{x} = 2,85 \pm 1,37$  ng/ml;  $n = 15$ ) en Desember ( $\bar{x} = 2,73 \pm 0,36$  ng/ml;  $n = 6$ ) en het relatief hoog gebly vanaf Augustus tot Januarie. Hierdie seisoenale patroon stem ooreen met die patroon wat in die Europese krimparkie (*Erinaceus europaeus*) waargeneem is.

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The South African hedgehog (*Atelerix frontalis*) has the ability to enter prolonged periods of torpor (Gillies, Ellison & Skinner 1991), and like other hedgehogs, may hibernate during winter in its natural environment. Saboureau, Castaing & Boissin (1984) illustrated that the European hedgehog will only hibernate after a dramatic decrease in plasma testosterone levels but that hibernation is not affected by castration and thus the physiological readiness to hibernate is probably determined by a rhythmic release of hormones into circulation. However, Fowler (1988a) showed that male and female hedgehogs readily become torpid under appropriate conditions during summer, even though males then have high levels of plasma testosterone.

Several studies on European hedgehogs (i.e. Courrier 1927; Allanson 1934; Mombaerts 1943; Girod & Curé 1965; Saure 1969) showed that testicular exocrine and endocrine functions are activated at the end of hibernation and that male hedgehogs in Europe have a seasonal cycle in testosterone production characterized by low levels in autumn and high levels from the end of winter to the middle of summer. In this regard no information has been published as yet on the South African hedgehog. The aim of the present paper is to describe seasonal changes in plasma testosterone levels in the South African hedgehog.

### Materials and Methods

#### Animals and blood collection

Fifteen male hedgehogs obtained from the National Zoological Gardens in Pretoria were kept in concrete enclosures on the experimental farm of the University of Pretoria (25°45'S / 28°12'E). Here they were subjected to natural photoperiod and temperature and were provided daily with food *ad libitum*. Heparinized blood samples (2,5 ml) were collected monthly from most of these males from February 1986 to February 1987 by cardiac puncture

after anaesthesia with fluothane (ICI South Africa [pharmaceuticals] Ltd). All samples were collected between 12h00–14h00 and plasma was stored at  $-20^{\circ}\text{C}$  until assayed.

#### Testosterone assay

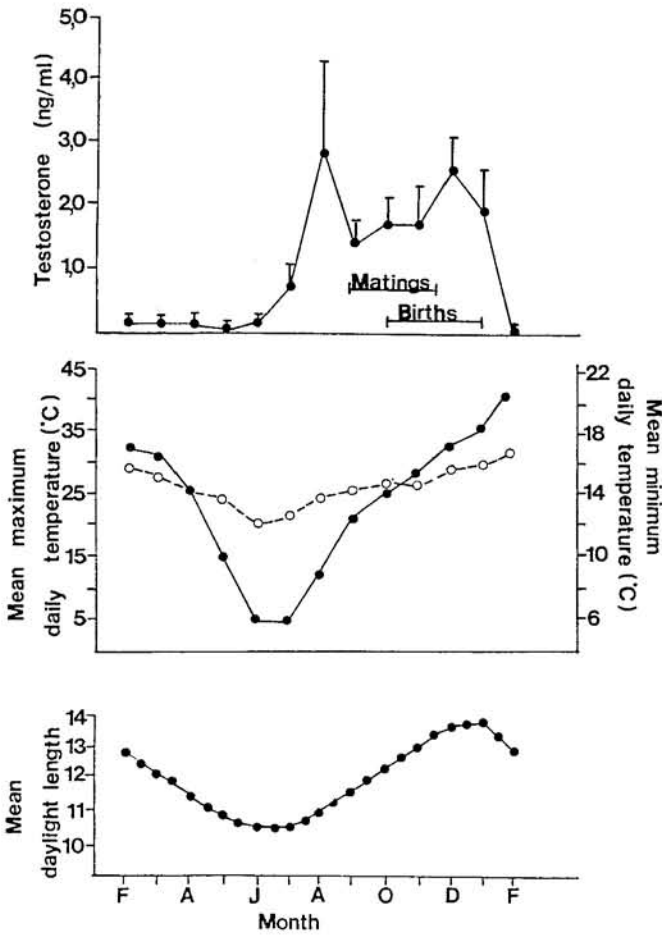
Plasma testosterone was measured by a radioimmunoassay of duplicate plasma aliquants using a procedure similar to that of Van Aarde & Skinner (1986). To denature sex steroid binding proteins (see Saboureau, Laurent & Boissin 1982) aliquants were mixed with 0,1 ml NaOH (0,6 M) and incubated at  $37^{\circ}\text{C}$  for 10 min before extraction (twice) with 4,0 ml diethyl ether (Merck, Darmstadt, FRG).

The antiserum was raised in a rabbit against testosterone-3-carboxymethyl-oxime conjugated to bovine serum albumin as described by Millar & Kewley (1976). Cross-reaction with all major naturally occurring steroids as determined by the supplier (R.P. Millar, Department of Chemical Pathology, University of Cape Town, RSA) was  $< 0,1\%$  except for dihydrotestosterone for which it was  $5,1\%$ . The sensitivity of the assays, defined as twice the standard deviation of the blank values ranged from 0,05–0,20 ng/ml (mean  $0,08 \pm 0,06\%$  ng/ml;  $n = 6$ ). Extraction efficiency as suggested by the recovery of labelled testosterone added to plasma varied from 82 to 91% (mean  $87 \pm 3,3\%$ ;  $n = 6$ ). Intra-assay coefficient of variation was 6,02%, and inter-assay coefficient of variation for a plasma sample containing 125 pg testosterone/ml was 18%. Testosterone values in serially diluted plasma followed the expected trend and were closely parallel to the standard curve.

All mean values in the text are followed by one standard deviation of the mean.

### Results

Mean plasma testosterone concentration remained low from February to June (0,05–0,24 ng/ml; Figure 1) but increased significantly ( $t = 3,77$ ;  $p < 0,01$ ) from July to August.



**Figure 1** Mean plasma testosterone levels in adult male hedgehogs, maximum daily temperature (○), minimum daily temperature (●) and mean daylight length recorded monthly from February 1986 to February 1987 (Vertical lines denote one standard deviation of the mean).

Testosterone levels peaked in August ( $2,85 \pm 1,37$  ng/ml;  $n = 15$ ) and December ( $2,73 \pm 0,36$  ng/ml;  $n = 6$ ). Levels remained high from August to January and the peak in August was followed by a non-significant ( $t = 2,75$ ;  $p < 0,01$ ) decrease in September ( $1,49 \pm 0,24$  ng/ml;  $n = 15$ ) and a gradual increase during October and November. The peak in December ( $2,73 \pm 0,63$  ng/ml;  $n = 6$ ) was not significantly higher than the mean value recorded for November ( $t = 1,58$ ;  $p < 0,01$ ). Matings ( $n = 3$ ) were recorded from the beginning of September to December and litters ( $n = 4$ ) were born between October and January.

Testosterone levels recorded during the period of increasing day length (July to December) were significantly higher ( $F = 29,45$ ;  $df = 1,120$ ;  $p < 0,01$ ) than those recorded during the period of decreasing day length (January to June). At the study site increasing day length was also associated with an increase in monthly maximum temperatures (Figure 1).

## Discussion

The seasonal changes in testosterone levels described in the present study are similar to those described in the related European hedgehog (*Erinaceus europaeus* L.). Absolute circulating levels of testosterone (0,05 to 2,73 ng/ml) in the

South African hedgehog were lower than those of European hedgehogs (0,56 to 20 ng/ml) but this might be ascribed to differences in the radioimmunoassay procedures and specificity of the antisera used to determine testosterone levels. The breeding season in *A. frontalis* extending from September to January, is characterized by high circulating testosterone levels with a decrease at the end of summer. Very low levels of testosterone were recorded from February to June, whilst testicular activity resumed at the end of winter (July to August) when the hedgehogs were still experiencing bouts of torpor.

In both European and South African hedgehogs the phenomenon of testicular recrudescence occurs at the end of winter during the hibernating period. The reproductive period can thus start once the cold season ends, as the onset of the reproduction depends on the time of spring arousal (Saboureau & Boissin 1983a). This is in agreement with the hypothesis of Pengelly & Asmundson (1974), who suggested that spring arousal may be an endogenous event caused by external conditions, and so become synchronized with those conditions. Indeed, Saboureau *et al.* (1984) later illustrated that the cycle of arousal was not influenced by external factors such as environmental temperature and food availability. By implanting silastic capsules filled with testosterone and releasing a hormonal level nearly equivalent to those measured during the reproductive season, they showed that hibernation is only possible after a dramatic decrease in plasma testosterone levels. Castration, however, modified neither the beginning of hibernation nor the resumption of locomotor activity (Saboureau *et al.* 1984).

In the present study testosterone values increased with an increase in daylight length maximum temperatures (Figure 1). It might be that an initial change in environmental factors serves both as a trigger or cue for an increased production of testosterone before the reproductive period, and likewise a decrease of testosterone production after the reproductive period. Fowler (1988b) concluded that the rate of change of the photoperiod has an important influence on male testosterone and body mass cycles of European hedgehogs while Saboureau & Boissin (1983a) showed that climatic factors can influence the sexual function and duration of reproduction in relation to latitude. It is also known that melatonin, *B*-endorphin and prolactin are important in the timing of reactivation of reproduction in the European hedgehog (Fowler 1988b). Although the present study, based upon total circulating testosterone levels illustrates a clear seasonal cycle in plasma testosterone in *A. frontalis*, Saboureau & Boissin (1983b) showed that the peripheral metabolism of testosterone and its metabolic clearance rate may also change seasonally. For this reason the additional factors involved in the seasonal patterns of testosterone recorded await further investigation.

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