ENDOCRINE CORRELATES OF THE ANNUAL REPRODUCTIVE CYCLE OF THE PORCUPINE *Hystrix africaeaustralis*

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Introduction

The *Cape porcupine Hystrix africaeaustralis* is a large (12-18 kg) nocturnal *hystricomorph rodent* occurring throughout southern Africa where it is sometimes used as a source of food and often regarded as a nuisance by crop-producing farmers. They live in family groups, these generally comprising an adult male, adult female and varying numbers of offspring of successive litters. Reproduction is characterized by a relatively long *oestrous cycle* (~30 days) and *gestation* period (94 days). Captive females breed throughout the year but free-ranging females in southern Africa only produce litters in summer when exposed to seasonal climatic changes (van Aarde, 1985a).

The present paper reports on seasonal changes in plasma *progesterone* and *oestradiol-17ß* levels in adult females and seasonal changes in plasma *testosterone* levels in adult males in an attempt to explain the annual reproductive pattern of porcupines.

Procedure

Materials and Methods

Females

-*Captivity*. Females housed with adult males in semi-outdoor concrete enclosures where they were exposed to natural conditions of illumination, ventilation and temperature, were immobilized and examined at 2-7 day intervals (van Aarde, 1985b).

The length of the oestrous cycle was taken as the interval from the first day of *vaginal opening* in one cycle up to, but not including, the first day of opening in the following cycle (Weir, 1974). Blood samples (5,0 ml) were collected through cardiac puncture between 10h00 and 12h00 and plasma was stored at -20°C. Gestation length was calculated as the interval between observed *copulation* and *parturition* for four females kept isolated from intact males after copulation. The lactation period was considered as the interval from birth to the day when milk could not be expressed from the nipples of immobilized females.

-*Free-ranging*. Ovaries and blood samples were collected from 54 adult females killed on the Tussen-die-Riviere Game Farm (TdR) (30° 25' S, 26° 12' E), South Africa, as part of a cropping programme. Their reproductive status was recorded and fetal age was determined using the formula of Huggett & Widdas (1951). Date of conception and date of birth were extrapolated from fetal age using a gestation period of 94 days. The age of each culled specimen was determined as described by van Aarde (1985c).

Males

Testes, epididymides, vesiculae seminales, prostate glands and blood samples were collected from 64 adult males killed over a one-year period on the TdR. Gonads and accessory glands were freshly weighed and testicular samples fixed in Bouin's fluid and stored in 70% ethanol were routinally prepared for microscopic examination. Sections were examined for the stage of *spermatogenesis* and *seminiferous tubule diameter* for each male was calculated as the mean of 25 tubules measured in cross-section. Plasma was stored at - 20°C until assayed. The age of each specimen was determined as described by van Aarde (1985c).

Radioimmunoassay of steroid hormones

-*Progesterone*. The procedure used was similar to that of Haresign *et al.* (1975). Plasma samples from pregnant females were, however, treated with 0,1 ml NaOH (0,6 ml/1) to denature progesterone binding plasma proteins (see Heap *et al.*, 1981) before extraction. Specificity of the antiserum (Specific Antisera Ltd, Wilmslow, U.K.) has been described by Furr (1973) and sensitivity of assays ranged from 0,16 to 0,73 ng/ml ($\bar{x} = 0,49 \pm 0,21$). Intra-and interassay coefficients of variation were 4,3 and 9,7% respectively. Extraction efficiency varied from 69,6 to 94,1% (van Aarde 1985d).

-*Oestradiol-17ß*. The procedure was similar to that of Abraham (1976) and specificity of the antiserum has been quantified by the supplier (R.P. Millar, Department of Chemical Pathology, University of Cape Town, South Africa). Cross-reactions of other steroids were < 0,01% and sensitivity of assays ranged from 3,7 - 15,8 pg/ml ($\bar{x} = 11,7 \pm 3,9$). Intra-and interassay coefficients of variation were 2,4 and 16,2% respectively. Extraction efficiency varied from 85,1 - 96,8% (van Aarde 1985d).

-*Testosterone*. The procedure was described by van Aarde (1984). Preparation of the antisera was described by Millar & Kewley (1976) and cross-reactions of all major naturally occurring steroids were < 0,01%, except for dihydrotestosterone for which it was 8,1%. Sensitivity of the assays ranged from 238 - 371 pg/ml ($\bar{x} = 29,1 \pm 70,4$). Recovery estimates varied from 89,1 to 92,8% ($\bar{x} = 91,2 + 1,9$) and intra-and interassay coefficients of variation were 5,3 and 13,6% respectively.

Results

Females in captivity

The oestrous cycle

Parous non-lactating females housed in captivity with intact adult males, experienced oestrus (perforation of the vaginal closure membrane) at 17 - 42-day intervals ($\bar{x} = 32,3 \pm 6,2$ days ; n = 24). Perforation of the vaginal membrane was associated with a surge in oestradiol-17ß secretion (peak values 25 - 176 pg/ml) and followed by an increase in circulating levels of progesterone, the latter attaining peak values (3,2 - 9,0 ng/ml ; $\bar{x} = 5,9 \pm 2,1$; n = 12) 8 - 19 days ($\bar{x} = 13,8 \pm 2,8$; n = 12) after the day of vaginal opening. The luteal phase of the cycle varied from 21 to 35 days ($\bar{x} = 29,3 \pm 2,6$; n = 8). Copulation occurred 2 - 8 days ($\bar{x} = 5,0 \pm 2,6$; n = 8) after vaginal opening and thus at the time when progesterone levels attained minimum values and oestradiol-17ß levels were on the decline. Nine parous females examined systematically over a one-year period, exhibited 81,5% of

the expected oestrous cycles and the number of cycles observed during the period of increasing daylight length as well as the period of decreasing daylight length did not differ significantly from that expected (χ^2 = 1,69 and 0,36 respectively).

Pregnancy

Pregnancy lasted 93 - 94 days. Progesterone levels during matings followed by conception, varied from 2,0 - 4,1 ng/ml ($\bar{x}$ = 3,2 ± 1,0 ; n = 3) and were significantly higher (t = -4,73 ; p < 0,01 ; d.f. = 7) than values observed (0,5 - 1,5 ng/ml ; $\bar{x}$ = 0,9 ± 0,5 ; n = 6) on days of 'sterile' matings (matings with intact males which were not followed by pregnancy). Oestradiol-17ß levels then varied from 22 - 34 pg/ml ($\bar{x}$ = 25,3 ± 7,6 ; n = 3).

Lactation

Lactation lasted 37 - 163 ($\bar{x}$ = 100,6 ± 37,8 ; n = 8) days and progesterone and oestradiol - 17ß levels remained at the limits of detection throughout the period of lactation. Oestradiol-17ß surges, accompanied by perforation of the vaginal closure membrane, followed by copulation and increases in progesterone levels (thus normal cyclic activity), occurred 2 - 42 days after the cessation of lactation. Females experienced 3 - 7 ($\bar{x}$ = 5,8 ± 2,1) post-lactational cycles before conceiving.

Litter interval

Females in captivity bred throughout the year with most litters (78,7% ; n = 165) being produced between August and March. This was significantly more than expected (χ^2 = 10,9 ; p < 0,05). Litter intervals recorded for eight females housed continuously with intact males varied from 296 - 500 days ($\bar{x}$ = 385 ± 60,4).

Free-ranging females

Females were reproductively active throughout the year. Extrapolation to the date of conception and birth, using specific fetal growth rates and fetal weights on the day of collection, indicate that matings between May and December were successful, resulting in a birth season from August to March. The birth peak in January coincided with peaks in mean monthly temperature and rainfall.

Males

Spermatogenesis, as suggested by the presence of spermatogonia, spermatocytes and spermatids in the seminiferous tubules of adult males (> 18 months old), occurred throughout the year. Combined testes weight and epididymides weight for adult males did not change seasonally. Prostate and vesicular glands were, however, significantly (t = 4,96 ; p < 0,001 ; d.f. = 32) heavier during the period of conception (May - December) than during the rest of the year. Mean seminiferous tubule diameter attained maximum values between May and December. The increase before May and decrease after December were, however, not significant (t = 1,43 and - 0,13 respectively ; d.f. = 11).

Seasonal changes in mean testosterone levels were not significant but increased from a nadir of 0,98 ± 0,44 ng/ml in January to 3,31 ± 2,11 ng/ml in March, with mean values remaining above 3,0 ng/ml until July.

Discussion

Porcupines are reproductively active throughout the year but free-ranging females conceived only between May and December, resulting in a birth season from August to March, this coinciding with the interval when most litters were produced in captivity. Seasonal breeding in the porcupine can not be attributed to seasonal oestrus. Cyclic activities, however, are affected by lactation which renders females anoestrous for three to four months each year. Lactational anoestrous is thus a limiting factor in female productivity. The physiological mechanisms underlying the 3-7 post-lactational cycles where copulation occurred without pregnancy following, are not known but the need for uterine priming for receptors may serve as an explanation. The sequence of events indicates that, following parturition, females invariably conceive at approximately the same time during the following season.

The full spermatogenic cycle observed in seminiferous tubules of all adult males suggests that males, in general, have the ability to fertilize ova throughout the year. The increased circulating levels of testosterone before the breeding season and the statistically significant heavier weights of prostate and vesicular glands during the breeding season, probably in response to the increased testosterone secretion, may be indicative of some degree of reproductive seasonality in males. This can, however, not as yet, be explained in terms of environmental or physiological variables. The inability to form a functionaly copulatory plug as a result of a decrease in accessory gland activity, may play a role in successful seasonal fertilization.

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