Circulating progesterone and oestradiol-17β concentrations in cyclic Cape porcupines, *Hystrix afercaeaualris*

R. J. van Aarde

*Mammal Research Institute, University of Pretoria, Pretoria 0002, South Africa*

**Summary.** The general pattern of steroid secretion during the 30-day oestrous cycle of the Cape porcupine is that of a surge (25-176 pg/ml) in oestradiol-17β secretion at the time of perforation of the vaginal closure membrane, followed by an increase in progesterone concentrations, the latter attaining peak values (mean 5·9 ± 2·1 ng/ml) 8-19 days (13·8 ± 2·8 days) after vaginal opening. Copulation occurred after the oestradiol-17β surge and the length of the luteal phase of the cycle varied from 21 to 35 days (29·3 ± 4·7 days), this representing 93% of the length of the cycle. Perforation of the vaginal closure membrane was not always accompanied by an increase in oestradiol-17β levels and some instances (19%) of vaginal opening were not followed by an increase in progesterone secretion. The hormonal characteristics of the oestrous cycle of females housed with vasectomized males were similar to those of females housed with intact males.

**Introduction**

There are no data available on the hormonal characteristics of the oestrous cycle of Old World hysticomorph rodents. Cape porcupine females (*Hystrix aflricaearialris*) are polyoestrous but do not experience oestrus while lactating and only conceive during the 3rd–7th cycle after the end of lactation (Skinner, van Aarde & van Jaarsveld, 1984). Reproductive processes in the porcupine conform to the general hysticomorph pattern (see Weir, 1974), including the presence of a vaginal closure membrane, a long gestation period (94 days), a long oestrous cycle (30 days) and the presence of numerous accessory corpora lutea (van Aarde, 1984). Porcupines are nocturnal, shelter and breed in burrows, rock crevices and caves, feed on bulbs, tubers, rhizomes and roots of a variety of plants and live in family groups comprising an adult male, adult female and a variable number of offspring. The present paper deals with temporal changes in circulating concentrations of progesterone and oestradiol-17β during the oestrous cycle of captive female porcupines.

**Materials and Methods**

*Animals*

The colony at the University of Pretoria's Experimental Farm was founded with animals donated by two zoological gardens and free ranging porcupines caught at various localities in South Africa. They were housed in groups of 2-5 in semi-outdoor concrete enclosures, fed daily on a mixture of fresh vegetables and fruit of the season and exposed to natural illumination, ventilation and temperature. Handling was effected by immobilization after an intramuscular injection of a mixture of 75 mg ketamine hydrochloride (Ketalar: Parke-Davis (Pty) Ltd, Isando, South Africa) and 20 mg xylazine hydrochloride (Rompun: Bayer, Johannesburg, South Africa).
Blood samples and examination

Blood samples (5·0 ml) were collected in heparinized tubes through cardiac puncture at 2–7-day intervals between 10:00 and 12:00 h from immobilized females and duplicate plasma fractions were stored at −20°C after separation by centrifugation. Females were weighed, the condition of the vaginal closure membrane was noted, and the teats were palpated to determine whether they were lactating. Following Weir (1974), 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. Enclosures where females were kept were inspected daily for the presence of copulatory plugs. All means are given with one standard deviation (s.d.) of the mean.

Radioimmunoassays

Progesterone. The procedure was, with minor modifications, similar to that of Haresign, Foster, Haynes, Crichton & Lamming (1975). Plasma was extracted by adding 2·0 ml petroleum ether (Saarchem (Pty) Ltd, Krugersdorp, South Africa) to duplicate plasma aliquants (0·05 or 0·1 ml) in glass tubes and thoroughly mixing the contents for 1 min on a vortex mixer. After freezing at −20°C for 60 min, the organic phase was decanted into a series of glass assay tubes (12 × 75 mm), and evaporated to dryness in a 37°C water bath. Dry extracts were dissolved in 0·1 ml phosphate buffer (pH 7·0) containing 0·1% gelatin and sodium azide by weight.

A series of standards containing 7·8, 15·6, 31·2, 62·5, 125, 250, 500, 1000 and 2000 pg progesterone/0·1 ml phosphate buffer was prepared in duplicate and included in each assay. Duplicate buffer blanks and dried residues of ether only, were included in each assay. Antiserum raised in a goat to progesterone-11-succinyl–bovine serum albumin as described by Furr (1973) and supplied by Specific Antisera Ltd (Wilmslow, U.K.) was added to plasma extracts, buffer blanks, ether blanks and standards at a 1:4000 dilution in phosphate buffer.

The mixture in each tube was incubated at room temperature for 10 min and after the addition of 0·1 ml (~20 000 c.p.m.) [1,2,6,7-3H]progesterone (Radiochemical Centre, Amersham, Bucks, U.K.) in phosphate buffer, the contents of the tubes were mixed for 1 min on a vortex mixer and incubated at 4°C for at least 12 h (usually overnight).

Progesterone bound to the antibody was separated from the free steroid by the addition of 1·0 ml dextran-coated charcoal (0·625% activated charcoal, Sigma, Dorset, U.K.; and 0·0625% dextran T40, Pharmacia, Uppsala, Sweden) at 4°C. The solutions were mixed for 30 sec, incubated at 4°C for 15 min and centrifuged at the same temperature at 1500 g for 15 min. The supernatants were decanted into scintillation vials and scintillation fluid (Ready Solve Hb/b: Beckman Instruments (Pty) Ltd, Johannesburg, South Africa) was added to each vial. The vials were shaken and the radioactivity was counted at least 4 h later for 2 min, using a Beckman LS 5800 Scintillation Counter.

The recovery of known amounts (~3000 c.p.m.)[1,2,6,7-3H]progesterone in ethanol to which 0·05 or 0·1 ml pooled plasma had been added served to determine procedural losses incurred during extraction.

Oestradiol-17β. The protocol was similar to that of Abraham (1976) and Crosignani, Trojsi, Attanasio, Lombroso Finzi & Malvano (1975). Steroids were not, however, purified and separated by chromatography after extraction.

The recovery of known amounts of [2,4,6,7-3H]oestradiol-17β (~1000 c.p.m.) Radiochemical Centre, Amersham, Bucks, U.K.) in absolute ethanol, dried under a stream of nitrogen, to which 1·0 ml pooled plasma from cyclic females was added, served to determine procedural losses incurred during extraction.

Plasma (1·0 ml), reagent blanks and tritiated steroid recovery samples were extracted with 4 ml diethyl ether (Merck, Darmstadt, FRG) by shaking the mixtures thoroughly for 5 min on a multitube vortexer (Model 2601, Scientific Manufacturing Industries, Emeryville, U.S.A.). The
aqueous phases were frozen at -20°C for 60 min and the ether extract decanted into a set of clean glass assay tubes (12 x 75 mm). These were evaporated to dryness under a stream of nitrogen in a water bath at 37°C. Dried extracts were resuspended in 0·1 ml phosphate buffer (pH 7·0) containing 0·1% gelatin and sodium azide by weight. A series of standards (0, 4·89, 9·75, 19·5, 39, 79, 156, 312, 625, 1250 and 2500 pg oestradiol-17β/0·1 ml phosphate buffer) was prepared in duplicate and included in each assay.

Antiserum in phosphate buffer (0·1 ml) at a dilution of 1:2500 was added to standards, plasma extracts and reagent blanks, vortexed and left to incubate at room temperature (23-25°C) for 10 min. [2,4,6,7-3H]oestradiol-17β (~10 000 c.p.m.) in 0·1 ml phosphate buffer was added to the contents of each tube which were vortexed again and left to incubate for about 12 h at 4°C (usually overnight).

The separation of antibody-bound and free steroid was performed at 4°C by adding 1·0 ml dextran-coated charcoal suspension to each tube. The contents were agitated for 1 min, incubated for 10 min at 4°C and centrifuged (1500 g) for 15 min at 4°C. Supernatants were decanted into scintillation vials and scintillation fluid was added to each vial. The contents of each vial were mixed thoroughly and radioactivity was measured in a liquid scintillation counter.

Duplicate samples (1·0 ml) from a pool of female porcupine plasma, and various quantities (25, 50 and 100 pg/ml) of oestradiol-17β added to a male plasma pool were included in each assay and served as internal controls.

Validations

**Progesterone.** The specificity of the antiserum has been described by Furr (1973) and cross-reactions of other steroids were: 11-hydroxyprogesterone, 85%; 17α-hydroxyprogesterone, 12·5%; 5β-pregnane-3,20-dione, 12·5%; 5α-pregnane-3,20-dione, 3·0%; 5β-pregnan-3β-ol-20-one, 1·73%; 11-deoxycorticoesterone, 1·1%; 5α-pregnane-3β-ol-20-one, 1·0%. Cross-reactions of 20α-hydroxyprogren-4-ene-3-one, 20β-hydroxyprogren-4-ene-3-one, 11-deoxycortisol, testosterone, androstenedione, pregnenolone, 5β-pregnan-3,20-diol and oestradiol-17β were less than 0·70%.

Sensitivity of the assays, defined as twice the standard deviation of blank values, ranged from 0·16 to 0·73 ng/ml (mean 0·49 ± 0·21 ng/ml; n = 7). Ten buffer blanks measured during the assays contained 0·46 ± 0·26 ng progesterone equiv./ml.

Recovery estimates varied from 81·0 to 94·1% (mean 86·6 ± 4·9; n = 7) for samples containing 0·5 to 5·0 ng progesterone/ml and from 61·1 to 77·2% (69·3 ± 4·4; n = 10) for samples containing >5·0 and <15·0 ng progesterone/ml plasma. Results were corrected accordingly. Intra- and interassay coefficients of variation calculated according to the method of Jeffcoate (1981) were 4·3 and 9·7% respectively. The interassay variation for a plasma sample containing 6·6 ng progesterone/ml included in all assays was 7·6%. Amounts of progesterone (5, 10 and 20 ng/ml) recovered from male plasma pools did not differ significantly from those expected.

**Oestradiol-17β.** The specificity of the antiserum raised in a rabbit against oestradiol-6-(O-carboxymethyl) oxime-bovine serum albumin conjugate has been quantified by the supplier (R. P. Millar, Department of Chemical Pathology, University of Cape Town, South Africa) and cross-reactions of other steroids were: oestrone, 0·01%, pregnanediol, corticosterone, deoxycorticosterone, 17α-hydroxyprogrenolone, androstenedione, 20α-dihydroprogesterone, progesterone, testosterone and cortisol, 0·001%.

Sensitivity of the assays, defined as twice the standard deviation of values obtained from buffer blanks, ranged from 3·7 to 15·8 pg/ml (mean 11·7 ± 3·9). Buffer blanks contained 8·3 ± 2·8 pg oestradiol-17β equiv./ml (n = 8). Extraction efficiency varied from 85·1 to 96·8% (mean 91·4 ± 4·3; n = 8) and results were corrected accordingly. The amount of oestradiol-17β (25, 50 and 100 pg/ml) recovered from male plasma pools did not differ significantly from that expected. Intra- and interassay variation was 2·4 and 16·2% respectively. Interassay variation for a sample containing 32·6 ± 3·1 pg/ml was 9·4%.
Text-fig. 1. Plasma progesterone and oestradiol-17β concentrations throughout the oestrous cycle of the porcupine. The arrows indicate observed day of opening of the vaginal closure membrane.

Results

Temporal changes in concentration of circulating progesterone and oestradiol-17β during 12 oestrous cycles monitored in 6 adult females are presented in Text-fig. 1. Cycles a, b, d, e and f were recorded in 2 parous females housed with a vasectomized male; Cycles c, g and j in 2 parous females, each housed with an intact male; Cycles h, i, k and l were in 2 nulliparous adult females.

Text-fig. 2. Plasma progesterone and oestradiol-17β concentrations in 2 parous female porcupines before, during and after perforation of the vaginal closure membrane. Black bars indicate the periods of vaginal opening and arrows denote the day of copulation.
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Text-fig. 3. Circulating progesterone and oestradiol-17β concentrations during the period after opening of the vaginal closure membrane in porcupines. Arrows indicate the day of observed vaginal opening.

Each housed with an intact male. Periods between 2 consecutive vaginal openings and therefore the assumed length of these oestrous cycles plus 1 day, varied from 23 to 42 days (mean 34 ± 6·6 days; \( n = 12 \)). The interval between oestradiol-17β surges did not differ significantly from the interval between consecutive instances of vaginal opening (33·4 ± 7·98 and 34·6 ± 6·8 days; paired \( t = 0·82; \) d.f. = 6; \( P < 0·05 \)). Oestradiol-17β surges coincided with vaginal opening.

Baseline and peak values of oestradiol-17β during the oestrous cycle varied within, as well as between females, with baseline values varying from the limits of detection of the assay (10 pg/ml) to 1.0 pg/ml. Measured peak values varied from 25 to 176 pg/ml. Copulation occurred after the oestradiol-17β surge (Text-fig. 2).

Progesterone rose to detectable levels (>0·5 ng/ml) 2–7 days after the observed day of vaginal opening and 6–12 days (mean 7·6 ± 1·8 days; \( n = 12 \)) after the observed peak in oestradiol-17β concentrations. Progesterone concentrations reached maximum values 8–19 days (mean 13·8 ± 2·8 days; \( n = 12 \); Text-fig. 1) after vaginal opening with peak values varying from 3·2 to 9·0 ng/ml (5·9 ± 2·1 ng/ml; \( n = 12 \)). The length of the luteal phase, as suggested by the time in days when progesterone concentrations were higher than 1·0 ng/ml, varied from 21 to 35 days (mean 29·3 ± 4·7 days; \( n = 12 \)).

Text-figure 3 presents oestradiol-17β and progesterone profiles during and after 14 instances of vaginal opening for which the lengths of the oestrous cycle either could not be defined or when vaginal opening was not accompanied by a rise in circulating levels of oestradiol-17β. Cycles a to k were observed in parous females housed with intact males and Cycles l, m, and n in nulliparous females housed under similar conditions. Ten of the observed instances of vaginal opening (Text-figs 3b, c, e, f, h, i, j, l, m and n) were not preceded, accompanied or followed by an increase
in oestradiol-17β values, this representing 38·5% of all the cycles monitored (including those presented in Text-fig. 1). Luteal activity, as suggested by an increase in circulating progesterone concentrations, did not follow vaginal opening in 5 instances (Text-figs 3 a, b, i, l and n), this presenting 19·2% of all the cycles monitored. Three of these occurred in 2 parous females and 2 in a nulliparous female.

Discussion

The presence of a vaginal closure membrane, which is usually only perforated at oestrus and parturition, provides a ready means for determining periodicity of the reproductive cycle of most hystricomorph rodents (see Weir, 1974). Synchronization of perforation with oestradiol-17β surges in 35% of the cycles monitored in porcupines confirms the suggestion of Rowlands & Weir (1977) that perforation of the membrane is an external indicator of ovulation. In the porcupine, surges in oestradiol-17β concentrations were nearly always followed by variable increases in circulating progesterone values. Some of the inconsistent profiles presented in Text-fig. 3 may be due to random sampling before, during or after opening of the vaginal closure membrane, while others may have resulted from ‘anovulatory’ cycles, early luteal regression or failure in the hormonal mechanisms responsible for the formation of the corpus luteum. Cyclic activities apparently were not affected by immobilization but may have had an effect on steroid concentrations.

Copulation always occurred after the peak in oestradiol-17β levels and the cyclic secretion of steroid hormones in females mated by vasectomized males (e.g. Text-figs 1a, b, d, e and f) was similar to that in females mated by intact males (i.e. Text-figs 1c, g and i). Cyclic activity in the porcupine is therefore not an artefact in females isolated from fertile males in captivity. Factors responsible for ‘sterile’ matings with intact fertile males are still unknown and nulliparous and parous females experienced 3–7 periods of oestrus before conceiving (van Aarde, 1984).

Circulating concentrations of progesterone during the oestrous cycle varied from 0·5 to 9·0 ng/ml and varied within as well as between females. Peak values (5·9 ± 2·1 ng/ml) were higher than those recorded in cyclic virgin guinea-pigs (2·8 ± 0·3 ng/ml; Challis, Heap & Illingworth (1971). Hossain, Lee, Clarke & O'Shea (1979) recorded peak values of 3·7 ± 1·1 ng/ml in cyclic guinea-pigs. Values recorded for the porcupine are lower than those recorded for nonpregnant cuis (Galea musteloides) (17·9 ng/ml; Tam, 1973).

Progesterone could be detected throughout most (approximately 93%) of the length of the cycle and attained maximum values midway through the cycle. Cyclic guinea-pigs attained maximum values on Day 12 of the 16-day cycle and declining luteal blood-flow did not initiate luteal regression (Hossain et al., 1979). The luteal phase of the oestrous cycle of the porcupine is therefore similar to that of the guinea-pig. The length of the cycle and the observed variation is, however, in agreement with that observed in some New World hystricomorph species and in the crested porcupine (see Weir, 1974).

Peaks in unconjugated oestradiol-17β concentrations in plasma varied from 25 to 176 pg/ml and occurred before copulation when progesterone levels were relatively low. In spite of the observed variability the general pattern of steroid secretion during the oestrous cycle is a surge in oestradiol-17β secretion at the time of perforation of the vaginal closure membrane, with progesterone concentrations increasing from basal values while or after oestradiol-17β levels decrease. Oestradiol-17β concentrations increase towards a peak when progesterone values decrease and no evidence exists for a progesterone surge on the first day of the cycle as has been observed by Blatchley, Donovan & ter Haar (1976) for guinea-pigs.

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References


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