

A preliminary investigation of the relationship between ovarian steroids, LH, reproductive behaviour and vaginal changes in lesser bushbabies (*Galago moholi*)

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Urinary hormone profiles, determined by means of radioimmunoassays, were aligned with changes in mating behaviour and vaginal morphology and cytology during the ovarian cycles of adult, female lesser bushbabies (*Galago moholi*), a prosimian species. Intromission occurred in all seven females, 2.0 ± 1.1 days (mean \pm SEM, median = 0) after the occurrence of vaginal opening, and lasted for 4.1 ± 0.7 days. Three females subsequently gave birth. Vaginal swelling and labial reddening were initiated at least 2.5 ± 0.5 days before vaginal opening and lasted 10.4 ± 0.9 days. Pro-oestrous and vaginal oestrous smears coincided with vaginal opening, specifically during the period of mating. Concentrations of immunoreactive oestradiol increased during the first few days of vaginal opening in one pregnant female and in the nonpregnant females, and coincided with mating. Concentrations of immunoreactive progesterone did not show any temporal pattern for either pregnant or nonpregnant females. Concentrations of immunoreactive testosterone were generally higher during vaginal swelling and opening than during the remainder of the cycle in one pregnant female, whereas in nonpregnant females, no specific temporal pattern was evident. In both pregnant and nonpregnant females, immunoreactive LH concentrations increased during the period of vaginal swelling and opening, while in nonpregnant females increases were also apparent after vaginal closure. The data reported here are preliminary, and further research is necessary to establish conclusively patterns of excreted hormones during the reproductive cycles of *Galago moholi* and in other prosimian species.

Introduction

The southern African lesser bushbaby (*Galago moholi*) is a prosimian primate, formerly regarded as a subspecies of the *Galago senegalensis* (lesser bushbaby) clade, but is at present considered a separate species (Olson, 1979); *G. moholi* is one of the smaller of the lesser bushbabies, with a mean adult body mass (in captivity) of 186 g for males and 163 g for females (Izard and Nash, 1988). Lesser bushbabies are nocturnal and arboreal and are generally non-gregarious, although several individuals may occupy overlapping home ranges and may share sleeping sites (Bearder and Doyle, 1974).

While there are several reports on *G. moholi*, concerning, for example, its seasonality, ovarian cyclicity, sexual behaviour, and gestation in the wild and in captivity (Bearder, 1969; Doyle *et al.*, 1967, 1971), no information is available on the relationship between reproductive endocrinology, vaginal morphology and cytology, and sexual behaviour during the ovarian cycle in this species.

Generally, in prosimians, research in the field of reproductive endocrinology has been carried out on only one species of Lorisidae (of which *G. moholi* is a member), namely, *Galago*

crassicaudatus (Eaton *et al.*, 1973), and on several lemurs, namely *Varecia variegatus* (Shideler *et al.*, 1983), *Lemur catta* (Von Horn and Resko, 1977; Bogart *et al.*, 1977) and *L. mongoz* (Perry *et al.*, 1992).

In a study on *G. crassicaudatus* (Eaton *et al.*, 1973), extended peak values of oestradiol coincided with a mean of 5.8 days of behavioural oestrus (mating behaviour), and fully cornified vaginal smears. After a fall in oestradiol concentrations (1–2 days before the end of female receptivity), a considerable increase in progesterone concentrations was apparent, which was maintained for much of the 24 day luteal phase. High concentrations of plasma progesterone and low concentrations of oestradiol at mid-cycle were correlated with sexual quiescence and a dioestrous smear (Eaton *et al.*, 1973).

In the Lemuridae, similar profiles of oestrogen and progesterone have been demonstrated, with a single periovulatory oestrogen peak lasting one day preceding one day of receptivity in *Varecia variegatus* (Shideler *et al.*, 1983), *Lemur catta* (Van Horn and Resko, 1977; Bogart *et al.*, 1977) and *L. mongoz* (Perry *et al.*, 1992), and a significant increase in progesterone following the post-ovulatory oestrogen decline during the dioestrous (luteal) phase in *L. catta* (Van Horn and Resko, 1977; Bogart *et al.*, 1977) and *L. mongoz* (Perry *et al.*, 1992).

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These changes in oestrogen and progesterone recorded during the ovarian cycles of female prosimians are similar to the patterns exhibited in the simians (Van Horn and Eaton, 1979).

Measurements of plasma concentrations of LH have previously been carried out in only two prosimian species, both belonging to the Lemuridae. In *L. catta*, an LH peak coincided with, or followed, the oestradiol peak by one day, and the subsequent increase in progesterone concentrations indicated that the surge of LH represented an ovulation event (Norman *et al.*, 1978). In another study on *L. catta*, LH immunoactivity could be demonstrated (Dunaif and Tattersall, 1991), while in *L. fulvus*, only small amounts of LH immunoactivity and no bioactivity were found (Dunaif and Tattersall, 1991). This result was paradoxical as *L. fulvus* showed higher concentrations of steroid hormones during the breeding season than did *L. catta* (Dunaif and Tattersall, 1991).

There is currently no information available on the pattern of androgen secretion in cyclic female prosimians. Plasma concentrations of testosterone in some female simians have been shown to reach peak values during the peri-ovulatory period (common marmosets: Kendrick and Dixon, 1983; owl monkeys: Bonney *et al.*, 1980; and rhesus monkeys: Hess and Resko, 1973). Reports on the role of testosterone in regulating behavioural patterns in certain species of simian are, however, conflicting (Dixon, 1983).

In *G. moholi*, for which no endocrine information is available, urinary concentrations of immunoreactive oestradiol, progesterone, testosterone and LH, and changes in vaginal morphology and cytology, and mating behaviour were monitored in cyclic females. Urinary hormone patterns have been demonstrated to reflect accurately reproductive events in many exotic mammals (see Hodges and Hearn, 1983) and in simians (Lasley *et al.*, 1980).

Materials and Methods

This study was approved by the Animal Ethics Screening Committee of the University of the Witwatersrand, Clearance Certificate No: 89/30/2.

Animals

Seven multiparous, cyclic females and seven adult, sexually experienced males were used. All animals were captive-born. The small sample size reflects the availability of the animals.

Housing

The animals were maintained in the Primate Behaviour Research Group Laboratory, University of the Witwatersrand, Johannesburg, South Africa. Apart from several behavioural observation cages (approximately 3 m × 2.5 m × 3.5 m), a holding room contained wire-mesh cages (70 cm × 50 cm × 75 cm), housing singly-caged experimental animals. The larger rooms contained ledges for nesting, food containers, branching systems, randomly placed, and vermiculite spread evenly across the floor. Visibility into each room was provided by a large one-way mirror, while outside, the observation area remained in total darkness. The temperature was controlled at 25 ± 1°C and relative humidity ranged from 54 to 61%.

The colony was maintained on an artificial day:night cycle (12 h light:12 h dark), with the dark phase (activity period) from 10:30–22:30 h. Artificial daylight, provided by white fluorescent tubes, alternated with red light supplied by white fluorescent tubes covered with transparent, red-coloured shades. Automatic timers controlled the changes in lighting conditions.

The animals were fed once daily with baboon cubes (Epol Ltd, Pretoria), fruits, nuts, an extra calcium supply (milk), occasionally mealworms and water *ad libitum*. The food was prepared and placed in each cage before the beginning of the activity period.

Vaginal morphology and cytology

Females were checked daily (between 08:00 and 10:00 h) for external vaginal changes. The first day of vaginal opening was designated as day 0. All behavioural vaginal morphological and cytological, and endocrinological events were considered in terms of the number of days before or after day 0. Durations of cycles were calculated from the day of vaginal opening to the day before the next occurrence of vaginal opening.

During the period of opening, vaginal smears were collected daily using a cotton swab and slides were stained with the Rapid Haematology Stain (Laboratory Reagents Service, Rietfontein) to determine cytological constituents of smears, including the presence of spermatozoa.

Vaginal cytology was categorized similarly to that of *G. crassicaudatus* (Eaton *et al.*, 1973): pro-oestrous smears contained mostly nucleated epithelial cells and smaller numbers of cornified epithelial cells. Vaginal oestrous smears comprised only cornified cells that were irregularly shaped, densely stained and maximally scattered. Metoestrous smears contained scattered and clumped cornified epithelial cells and string mucus, and sometimes leucocytes. Dioestrous smears consisted of non-cornified epithelial cells, string mucus and large numbers of leucocytes.

Behavioural observations

Pair-tests were carried out daily (13:00–16:00 h) for 26–42 days (mean = 32.4 days), approximating one ovarian cycle. In six pairs, this coincided with one period of vaginal opening, and in the seventh pair with two consecutive periods of vaginal opening.

Each female was tested with the same male throughout the study; familiarity had previously been established. Before the test, the female was transported in a wooden box (30 cm × 25 cm × 25 cm) from the holding room to the testing cage which housed the male. The pair was observed for 30 min. For the duration of the tests, the female was unable to physically interact with any other individual, and the male was completely isolated from all other animals.

Urine collection

Urine was collected in preference to blood samples because of the small size of the animals (150–200 g) and to obviate stress, which may adversely affect hormonal patterns. Samples were collected daily for the duration of behavioural tests from each female between 09:00 and 11:00 h, before commencement of the activity phase (11:30 h). Females were placed within two

apposed sieves situated over a removable collecting container. If the female failed to urinate, she was released into a holding cage with a plastic-coated cardboard tray as the substrate. The latter proved to be a more effective and less disruptive means of urine collection. Urine was aspirated into a sterilized vial and stored frozen at -20°C until assayed. Urine was not voided in one of the pregnant females (no. 3), possibly owing to stress.

Hormone assays

Urinary hormone radioimmunoassays were carried out in two of the three pregnant females, and in the five nonpregnant females (one of which became pregnant in the following cycle). In the absence of conclusive identification of the hormone measured in each assay, concentrations must be considered in terms of hormone immunoreactivity.

Oestradiol. Concentrations of immunoreactive oestradiol were measured in urine extracts (using diethyl ether) by radioimmunoassay following the procedure of Abraham (1976). Details of the assay set-up can be found in Bennett (1994). The specificity of the antiserum was quantified by R. P. Millar (Department of Chemical Pathology, University of Cape Town). Crossreactions with all major naturally occurring steroids (pregnanediol, corticosterone, deoxycorticosterone, 17α -hydroxypregnenolone, androstenedione, 20α -dihydroprogesterone, progesterone, testosterone and cortisol) were $< 0.01\%$, except oestrone which was 0.05% .

The detection limit of the assays, calculated from the amount of hormone required to suppress the binding of the labelled hormone to 90% of the binding achieved in the absence of the unlabelled hormone was 0.37 ± 0.23 pg per $100 \mu\text{l}$ (mean \pm SD), ($n = 7$). Recovery estimates were $84 \pm 6.9\%$ ($n = 6$). Results were corrected accordingly. The intra-assay coefficient of variation was 2.6% ($n = 2$), while the interassay coefficient of variation was 12.0% ($n = 8$).

Progesterone. The procedure was similar to that of van Aarde (1985), with minor modifications. Extraction from urine samples (0.1 ml) was accomplished using 6 ml petroleum ether at 40 – 60°C (BDH Chemicals Ltd, Poole, Dorset) on a horizontal shaker for 10 min. After freezing, decanting and drying down the supernatant, the dried extracts were reconstituted in 0.4 ml benzene (BDH Chemicals Ltd).

The specificity of the antiserum was quantified by R. P. Millar. Crossreactions with other steroids were: 11β -hydroxyprogesterone, 47.1% ; 11α -hydroxyprogesterone, 25.8% ; 5α -pregnane- $3,20$ -dione, 24.8% ; pregnenolone, 3.1% ; 11 -deoxycorticosterone, 2.2% ; 17α -hydroxyprogesterone, 1.9% ; 11 -deoxycortisol, 1.5% ; 3α -hydroxy- 5 -pregnane- 20 -one, 20α -hydroxy- 4 -pregnane- 3 -one, 0.4% ; cortisol, $< 0.2\%$; testosterone, 4 -androstenedione, oestradiol and oestrone, $< 0.001\%$.

The detection limit of the assays (as determined for oestradiol) was 0.23 ± 0.04 ng per $100 \mu\text{l}$ ($n = 7$). Recovery estimates were 100% ($n = 7$). The intra-assay coefficient of variation was 4.5% ($n = 4$), and the interassay coefficient of variation was 8.6% ($n = 9$).

Testosterone. A similar procedure to the oestradiol assay was adopted for testosterone with some modifications. Extrac-

tion from urine samples (0.1 ml) was achieved using 0.1 ml ammonia solution (25%) and 6 ml Aldrich diethyl ether (BDH Chemicals Ltd). The specificity of the antiserum was quantified by R. P. Millar. Crossreactions with other steroids were: 17β -hydroxy- 5α -androstane- 3 -one (DHT), 100% ; 4 -androstene- $3,17$ -dione (androstenedione), 5.1% ; 3 -hydroxy- 5 -androstene- 17 -one (dehydroepiandrosterone), 3β -hydroxy- 5α -androstane- 17 -one (etiocolanone), 3α -hydroxy- 5α -androstane- 17 -one (androsterone), progesterone and oestradiol, 0.008% .

The detection limit of the assays (as determined for oestradiol) was 0.07 ± 0.003 ng per $100 \mu\text{l}$ ($n = 7$). Recovery estimate was $91.1 \pm 5.1\%$ ($n = 5$). The intra-assay coefficient of variation was 3.7% ($n = 4$). The interassay coefficient of variation was 14.9% ($n = 7$).

Luteinizing hormone (LH). The immunoradiometric assay (IRMA) LH MAIAclone (Serono Diagnostics Ltd, Woking, Surrey) kit was evaluated for the measurement of LH in urine samples of lesser bushbabies. This commercial kit uses two high affinity monoclonal antibodies (sandwich technique) and achieves separation with a magnetic solid phase (MAIA).

The recommended assay protocol was adhered to and was as follows: 0.1 ml aliquots of standards (0.5 , 2 , 10 , 25 , 100 , 200 mIU ml^{-1} in bovine serum with sodium azide (0.2% w/v)), controls and urine samples were pipetted into propylene tubes. 0.1 ml of ^{125}I -labelled anti-LH reagent (fluorescein and ^{125}I -labelled mouse monoclonal antibodies to LH, < 8 Ci per vial, in phosphate buffer, containing normal sheep serum, BSA, inert dye and sodium azide (0.2% w/v)) was added to all tubes which were then vortexed for 30 s and left to incubate for 1 h at room temperature (22 – 25°C). LH MAIAclone Separation Reagent (0.1 ml, containing sheep antiserum to fluorescein covalently bound to magnetic particles in a Tris buffer with BSA and sodium azide (0.1% w/v)) was pipetted into all tubes, vortexed for 30 s and incubated at room temperature for 5 min. The rack of tubes was inserted into the MAIAclone Magnetic Separator for 2 min. The supernatant was decanted and 0.5 ml MAIAclone Wash Buffer (containing Tris buffer with sodium azide (0.8% w/v)) was added to all tubes and vortexed for 30 s. The tubes were left for 2 min at room temperature after which they were inserted into the magnetic separator for 2 min. After decanting the supernatant, the radioactivity was counted for 2 min using a gamma counter.

The specificity of the MAIAclone assay system was determined by the suppliers. Crossreactions with other gonadotrophic hormones were: hTSH, 19% ; hCG, 0.3% ; hFSH, $< 0.001\%$; hGH, $< 0.0001\%$.

The detection limit of the assay was 0.15 mIU ml^{-1} . The intra-assay coefficient of variation was 4.7% ($n = 2$) and the interassay coefficient of variation was 7.3% ($n = 4$).

Creatinine determination. Urinary creatinine concentrations were determined by the Jaffe reaction (Bonsnes and Tausky, 1945), which accounts for variations in the concentration of hormone in different volumes of urine obtained over the collection period. Concentration of hormone was expressed as

mass of hormone per mg creatinine. The intra- and interassay coefficients of variation for the creatinine control were 2.9% ($n = 2$) and 4.8% ($n = 18$), respectively.

Results

Vaginal morphological and cytological changes

The mean duration of the cycle calculated for six females (eight cycles) was 38.5 ± 2.1 days (mean \pm SEM), range 30–46 days. In all females, vaginal swelling and reddening of the labia preceded vaginal opening by 1–5 days (2.5 ± 0.5 days, mean \pm SEM; $n = 9$ cycles). However, vaginal swelling may have begun before the first day of sampling in some females. The vagina remained fully open for 8–14 days (10.4 ± 0.9 days; $n = 9$ cycles) in all females, during the latter part of which the orifice sometimes contained thick white mucus. This was followed by a flattening and constriction of the orifice and labia. Thereafter the vagina gradually closed, eventually reverting to an imperforate condition.

In pregnant females, the first indication of the vagina closing (medium vaginal opening), occurred on day 10 (day 9.7 ± 2.4 , $n = 3$), and further closure (small opening) on about day 15 ($n = 2$, no data for one of the females) after the initiation of vaginal opening. In nonpregnant females, vagina closure (medium vaginal opening) was initiated on day 8 (day 8.0 ± 2.1 , $n = 3$), and further closure (small opening) on day 11 (day 11.0 ± 2.6 , $n = 3$) following the first day of vaginal opening.

Pro-oestrous smears coincided with the first day of vaginal opening in all females. However, female no. 2 displayed a vaginal oestrous smear on the first day of opening in the first cycle. Vaginal oestrous smears were initiated on day 1 (day 1.3 ± 0.3 , $n = 3$) of vaginal opening in pregnant females and day 3 (day 2.7 ± 0.9 , $n = 3$) of opening in nonpregnant females. Metoestrous smears were first observed on day 6 of vaginal opening in pregnant (day 6.0 ± 1.0 , $n = 3$) and nonpregnant (day 6.3 ± 0.9 , $n = 3$) females, and dioestrous smears on approximately day 8 of vaginal opening in pregnant (day 7.7 ± 0.9 , $n = 3$) and nonpregnant (day 8.3 ± 1.5 , $n = 3$) females.

Mating behaviour

A typical behavioural sequence was initiated by the male who approached, contacted, and sniffed the genitals of the female. Occasionally, the pair engaged in naso–naso contacts (muz–muzs). The female immediately withdrew and was chased by the male through a series of approaches and withdrawals until contact occurred or the male stopped chasing. While in close proximity, the pair sometimes allo-groomed.

For the remainder of the test period, behavioural interactions alternated with investigation and marking of the environment, feeding and resting by both individuals. Although the male initiated most interactions, the female sometimes approached, contacted, sniffed and groom-solicited him. During these tests the male was never observed to display any behaviour indicative of sexual interest.

There were notable changes in the male's behaviour during the period of vaginal swelling, with increases in male genital sniffs and licks, and the initiation of male grabs, unsuccessful mounts, mounts and penile thrusts and female presents. There was an even greater increase in these behaviours during vaginal opening, while genital licks were more protracted and intromissions were newly manifested.

During a normal mating bout, the male mounted the female and grasped her around the waist with his forelimbs. He attempted to intromit with rapid 'searching' thrusts (as described by Eaton *et al.*, 1973, in *G. crassicaudatus*). If unsuccessful, he dismounted and licked the female's genitalia. This sequence was repeated until intromission was attained. After the termination of mating, both individuals carried out auto-genital licking and general self-grooming.

Behavioural patterns exhibited by the female at this time were overshadowed by the male's persistence in attempting to copulate with the female. However, during behavioural oestrus, she sometimes approached the male and presented her rear towards him by arching her back and raising her tail. After a male mount and during intromission, the female often made frequent loud vocalizations and attempted to spat or bite the male.

Intromission was initiated on the first day of vaginal opening in all three pregnant females and in two of the four nonpregnant females (Table 1). The duration of the mating period was slightly shorter, but the inter-individual variation was lower, in pregnant females (3.7 ± 0.3 days; $n = 3$) than in nonpregnant females (4.4 ± 1.1 days; $n = 5$).

Spermatozoa were observed in the vaginal smears of all three pregnant females from the first day of intromission (first day of vaginal opening) for 3.7 ± 0.3 days, while spermatozoa were found in the vaginal smears of three of the five nonpregnant females from the first ($n = 2$ females) and second ($n = 1$ female) days of intromission, for 3.0 ± 1.0 days (Table 1).

The average duration of gestation, calculated from the first day spermatozoa were present in vaginal smears up to the day of parturition, was 129.0 ± 1.8 days ($n = 3$) (Table 1).

Hormone profiles

Temporal changes in concentrations (mean \pm SEM) of immunoreactive oestradiol, progesterone, testosterone and LH in one pregnant (Fig. 1) and five nonpregnant females (Fig. 2), are shown. The low sample size precluded a statistical appraisal of hormonal changes between nonpregnant and pregnant females. Missing values on a number of cycle days was due to non-voiding of urine, or to insubstantial urine volumes collected for assaying.

It was not possible to construct a composite hormone profile for the two pregnant females, so each will be dealt with individually.

In female 1, peak concentrations of immunoreactive oestradiol ($246 \text{ pg mg}^{-1} \text{ Cr}$) and LH ($0.3 \text{ mIU mg}^{-1} \text{ Cr}$) were recorded on day 2 of vaginal opening compared with concentrations of $\leq 100 \text{ pg mg}^{-1} \text{ Cr}$ (Fig. 1a) and $\leq 0.1 \text{ mIU mg}^{-1} \text{ Cr}$ (Fig. 1b), respectively, on day 3. However, there was a slight increase in oestradiol concentrations ($144 \text{ pg mg}^{-1} \text{ Cr}$) on day 8 of opening.

Table 1. Mating data for female *Galago moholi*

Female number	First day of mating ^a	Duration of mating (days)	First day spermatozoa present in smear ^a	Time spermatozoa present in smears (days)	Gestation (days)
Pregnant					
1	0	4	0	4	132
2b ^b	0	4	0	4	129
3 ^c	0	3	0	3	126
Mean \pm SEM	0	3.7 \pm 0.3	0	3.7 \pm 0.3	129 \pm 1.8
Nonpregnant					
2a ^b	0	1	0	1	—
4	0	5	1	4	—
5	6	3	—	—	—
6	8	7	—	—	—
7b	2	6	2	4	—
Mean \pm SEM	3.2 \pm 1.6 ^d	4.4 \pm 1.1	1.0 \pm 0.6	3.0 \pm 1.0	—
Combined Mean \pm SEM	2.0 \pm 1.1 ^e	4.1 \pm 0.7	0.5 \pm 0.3	3.3 \pm 0.5	—

^aDay 0 is the first day of vaginal opening; ^bfemale 2 was monitored for two consecutive periods of vaginal opening, 2a and 2b; ^cno hormone measurements possible in this female; ^dmedian is 2.0; ^emedian is 0.

Concentrations of immunoreactive progesterone remained below 30 ng mg⁻¹ Cr during the time of vaginal opening, but increased on day 14 (46 ng mg⁻¹ Cr) (Fig. 1c).

Concentrations of immunoreactive testosterone increased rapidly on day -2 (1.8 ng mg⁻¹ Cr) and reached peak values on day 2 (2.9 ng mg⁻¹ Cr) compared with concentrations \leq 1.3 ng mg⁻¹ Cr from day 3 onwards (Fig. 1d).

In contrast, in the other pregnant female (no. 2), oestradiol and testosterone concentrations were depressed ($<$ 40 pg mg⁻¹ Cr and about 1 ng mg⁻¹ Cr, respectively) during vaginal opening. LH concentrations were raised before vaginal swelling at about 0.2 mIU mg⁻¹ Cr and increased to 0.4 mIU mg⁻¹ Cr on day -1 of opening. Only two measurements for LH were obtained during vaginal opening: day 1 (0.2 mIU mg⁻¹ Cr) and day 4 (0.04 mIU mg⁻¹ Cr). Concentrations of progesterone were consistently low (\leq 30 ng mg⁻¹ Cr). However, few measurements could be obtained from this female during the period of testing because of the lack of urine samples.

Although the composite hormone profiles in the five non-pregnant females displayed large inter-individual variation, certain patterns were evident, mainly in oestradiol profiles. Concentrations of oestradiol during vaginal swelling and the first day of vaginal opening ranged between 97 and 107 pg mg⁻¹ Cr (mean = 103 pg mg⁻¹ Cr). On days 1-3 of vaginal opening, oestradiol increased to concentrations of between 140 and 175 pg mg⁻¹ Cr, after which it declined gradually to lower concentrations ($<$ 75 pg mg⁻¹ Cr) from day 8 of vaginal opening (Fig. 2a). Increased oestradiol concentrations on days 1-3 of vaginal opening coincided with mating behaviour in most nonpregnant females.

In spite of large inter-individual variations, mean LH concentrations were raised on day -2 (0.3 \pm 0.1 mIU mg⁻¹ Cr) and days 0-3 (between 0.2 and 0.3 mIU mg⁻¹ Cr). While mean LH concentrations were generally lower from day 4 ($<$ 0.1 mIU mg⁻¹ Cr), increased concentrations were recorded on days

11-12 (approximately 0.2 mIU mg⁻¹ Cr) and days 15-16 (\approx 0.3 mIU mg⁻¹ Cr) (Fig. 2b).

There was no obvious temporal pattern in mean concentrations of progesterone in the nonpregnant females, although mean concentrations were slightly lower from day -3 to day 2 of vaginal opening (22-32 ng mg⁻¹ Cr) compared with 56 ng mg⁻¹ Cr on day -7 and 59 ng mg⁻¹ Cr on day 14 of opening (Fig. 2c).

It was not possible to establish any specific pattern in mean testosterone concentrations in nonpregnant females, although values declined on day 5 (0.6 \pm 0.3 ng mg⁻¹ Cr, n = 3), day 11 (0.8 \pm 0.2 ng mg⁻¹ Cr, n = 4) and days 15-18 (between 0.7 and 1.0 ng mg⁻¹ Cr) (Fig. 2d).

An integration of the hormonal, vaginal and mating behavioural data was possible in only one pregnant female. Successful copulations, that is, the presence of spermatozoa in vaginal smears, coincided with vaginal opening, pro-oestrous and vaginal oestrous smears, and increased concentrations of oestradiol, LH and testosterone on the third day (day 2) of vaginal opening (Fig. 3).

Discussion

The mean cycle duration of 38.5 days obtained in the present study is longer than the means of 31.7 and 32.9 days given for *Galago senegalensis* by Manley (1966) and Darney and Franklin (1982), respectively, and may reinforce the distinction between the two species. This distinction may be also true for the duration of vaginal opening (= oestrus, see Darney and Franklin, 1982) which was longer in *G. moholi* (mean = 8.4 days) in the present study compared with 5.4 days in *G. senegalensis* (Darney and Franklin, 1982).

In the present study, changes in vaginal morphology and cytology, duration of the mating period and the presence of spermatozoa in smears appear similar in pregnant and nonpregnant females. However, pregnant females displayed less

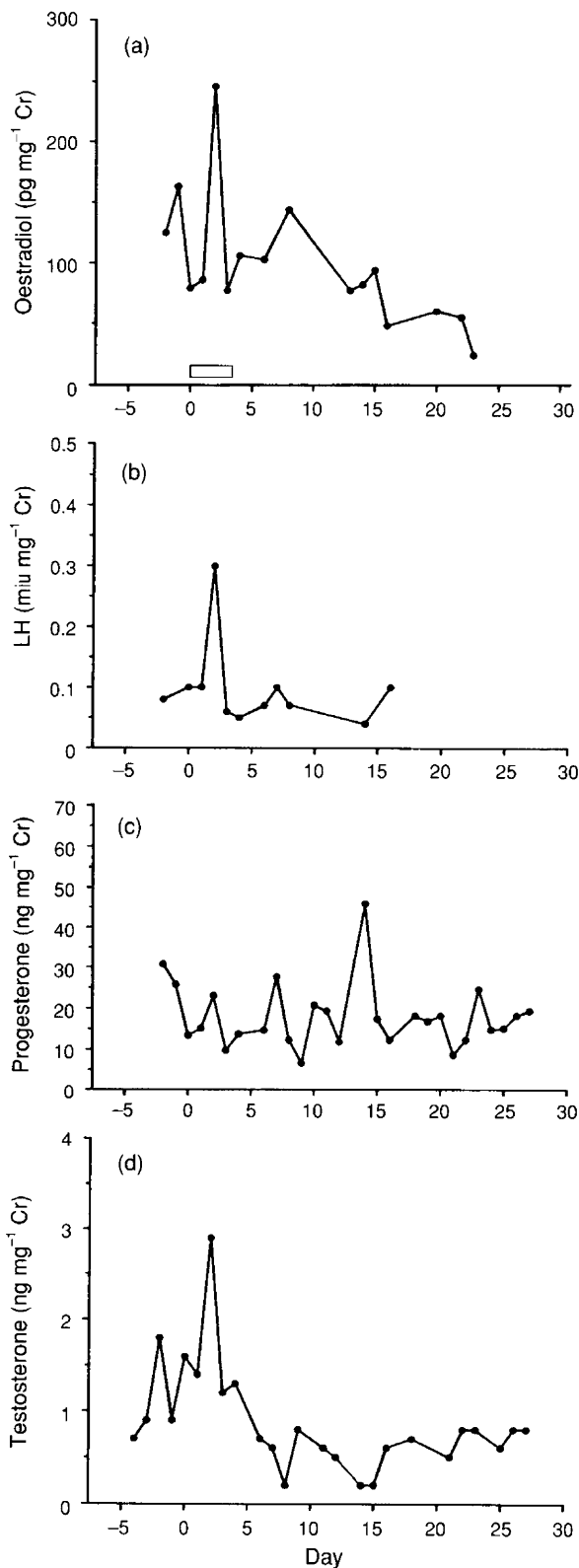


Fig. 1. Temporal patterns of urinary concentrations of immunoreactive (a) oestradiol, (b) LH, (c) progesterone and (d) testosterone during the ovarian cycle of a pregnant female lesser bushbaby (*Galago moholi*). Concentrations of hormones are expressed as mass of hormone mg^{-1} Cr. Day 0 = first day of vaginal opening; \square : intramission.

variability in the duration of the mating period and in the time when spermatozoa were present in vaginal smears than did nonpregnant females.

Changes in behavioural patterns during the cycle for *G. moholi* in the present study appear similar to those observed by Doyle *et al.* (1967). However, Doyle *et al.* gave no indication of the overt behavioural change in females during the period of mating, such as female approaches and occasional presentations, observed in the present study. In spite of these female behavioural manifestations, courtship was almost always initiated by the male in the present study, as documented by Doyle *et al.* (1967).

Since female crouch postures that indicate receptivity in *L. catta* (Evans and Goy, 1968) and *G. crassicaudatus* (Eaton *et al.*, 1973) were not observed in *G. moholi* in the present study, it was difficult to establish a behavioural component of female receptivity in this species. However, as in the study of Eaton *et al.* (1973), days on which intromission was achieved were classified as behavioural oestrus, and this was considered as an indication of receptivity in the present study. Female non-receptivity was suggested by unsuccessful mounting attempts by the male which continued for 1–2 days after the last day of intromission.

A mating period of approximately 4 days observed in the present study is similar to the 1–3 days obtained by Doyle *et al.* (1967). While this may be a feature of reproduction of lesser bushbabies in captivity, in the wild, the mating period may last only one day (Bearder and Martin, 1979). Eaton *et al.* (1973) have suggested that the separation of the pair before ejaculation may account for the extended period of mating in *G. crassicaudatus*; however, separation did not occur in either the study by Doyle *et al.* (1967) or the present study. Other factors may be responsible for extending the mating period in captivity, such as a constant photoperiod.

The observation of spermatozoa in the smears of three females that did not give birth suggests that these matings were sterile, since one of the females gave birth after mating during the next cycle, while the other females remained cyclic. It is probable that the male did not ejaculate in matings in which spermatozoa were not found in vaginal smears, since smears were collected immediately after pair-testing, and those collected after successful copulations consistently displayed large numbers of spermatozoa.

In the present study, the increase in mean concentrations of immunoreactive oestradiol during the period of vaginal opening, coinciding with mating behaviour in one pregnant female and in most nonpregnant females in *G. moholi*, was similar to the coincidence of high plasma concentrations of oestrogen and mating for an extended period in *G. crassicaudatus* (Eaton *et al.*, 1973). In contrast, in *L. catta*, oestradiol reached peak values on one day and was followed on the next day by a single day of mating (Van Horn and Resko, 1977). These observations suggest that there is a positive relationship between the duration of raised oestradiol concentrations and the duration of the mating period in the two species. Further research on a larger number of prosimian species is necessary for this to be verified.

The absence of a temporal pattern of mean concentrations of immunoreactive progesterone in *G. moholi* in the present study, in contrast to increasing post-ovulatory values in *G. crassicaudatus* (Eaton *et al.*, 1973) and *L. catta* (Van Horn and Resko,

1977), suggests that in urine, unlike plasma, a progesterone metabolite may be more representative of changes in female reproductive physiology. For instance, the urinary

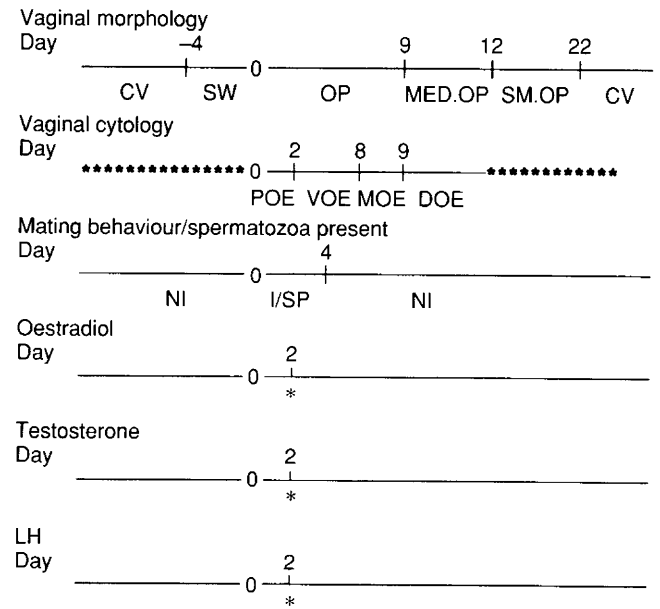
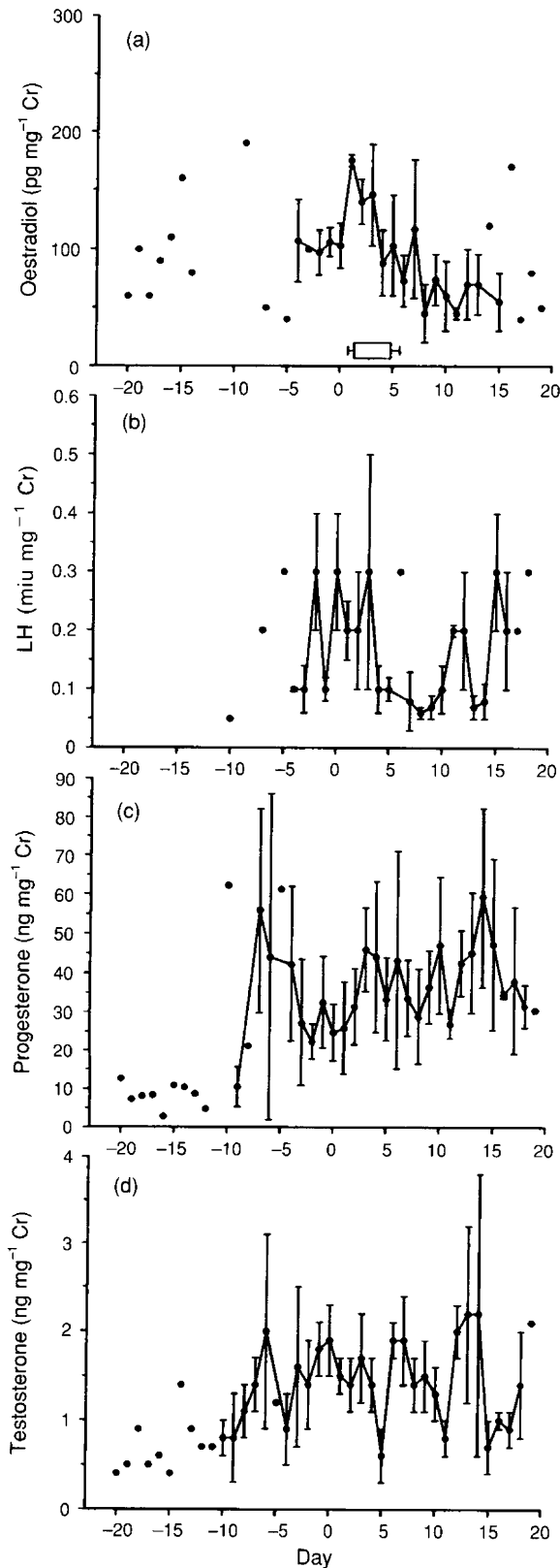


Fig. 3. Schematic representation of the timing of mating behaviour, vaginal changes and (*) increased concentrations of immunoreactive oestradiol, LH and testosterone during the ovarian cycle of a pregnant female lesser bushbaby (no. 1) (*Galago moholi*) (not drawn to scale). CV: closed vagina; SW: swollen vagina; 0: first day of vaginal opening (day 0); OP: open vagina; MED.OP: medium vaginal opening; SM.OP: small vaginal opening; POE: pro-oestrous smear; VOE: vaginal oestrous smear; MOE: metoestrous smear; DOE: dioestrous smear; ***: smear collection not possible; I/SP: intromissions and sperm in vaginal smears; NI: no intromissions.

progesterone metabolite, pregnanediol-3 α -glucuronide, has been effectively used to monitor changes in the ovarian cycle of owl monkeys (Bonney *et al.*, 1979).

The measurement of immunoreactive testosterone in this study is the first documentation of the pattern of excretion of this hormone in female prosimians. The increase in mean testosterone concentrations during vaginal swelling and on day 2 of opening in one pregnant female corresponded to the mid-cycle (peri-ovulatory) peak that occurs in higher primates (Bonney *et al.*, 1980; Hess and Resko, 1973; Kendrick and Dixson, 1983). However, it is uncertain what the effects of higher concentrations of testosterone during oestrus are on female behaviour, since oestrogen *per se* appears sufficient for the manifestation of female receptivity (see Lipschitz, 1994).

In the present study, although there was wide inter-individual variability in mean immunoreactive LH concentrations, its increase on the third day (day 2) of vaginal opening in the one pregnant female agreed with an estimation of the

Fig. 2. Temporal patterns of urinary concentrations (mean \pm SEM) of immunoreactive (a) oestradiol, (b) LH, (c) progesterone and (d) testosterone during the ovarian cycles of nonpregnant female lesser bushbabies (*Galago moholi*) ($n = 5$). Concentrations of hormones are expressed as mass of hormone mg⁻¹ Cr. Day 0 = first day of vaginal opening; \square : intramission. Individual plots are used when the number of samples that could be obtained were insufficient for statistical evaluation.

timing of ovulation on the second or third day of female receptivity in *G. crassicaudatus* (Eaton *et al.*, 1973) and was close to its timing on the second day of vaginal opening in *G. senegalensis*, where it was based on the recovery of haemorrhagic, preovulatory size follicles (Darney and Franklin, 1982).

The overlap of mean immunoreactive oestradiol and LH peaks on day 2 of vaginal opening in one pregnant female in the present study was similar to an LH peak coinciding with, or following, oestradiol peaks by one day, in *L. catta* (Norman *et al.*, 1978). Eaton *et al.* (1973) suggested that ovulation occurs on the day after the oestradiol peak in *G. crassicaudatus*.

Although immunoreactive LH displayed an increase coinciding with raised immunoreactive oestradiol concentrations during the period of vaginal swelling and opening, and a decline from day 4 of opening in nonpregnant females, it is uncertain why further increases occurred several days later, during the period of vaginal closure.

The results in the present study provide preliminary information of sex hormone and LH changes and their relationship with reproductive behaviour and physiology during the ovarian cycle of female *G. moholi*. However, it is clear that further research is required to establish unequivocally, endogenous hormone profiles, in this and other prosimian species.

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