Social influences on oestrous cycle length and plasma progesterone concentrations in the female lesser mouse lemur (*Microcebus murinus*)

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**Summary.** Plasma progesterone concentrations were recorded during one breeding season in 19 lesser mouse lemur females living in different social conditions. The oestrous cycle length and the progesterone profile mainly depended on the social environment of the female. For totally isolated females, the oestrous cycle lasted 38 ± 5.7 days and included a 25-30-days spontaneous luteal phase with a progesterone peak about 100 ng/ml between the 20th and 25th days after oestrus, and a prolonged preovulatory period of 10-15 days which could be considered equivalent to the follicular phase of a menstrual cycle. When females were able to communicate through olfactory, visual and auditory signals, the oestrous cycle was significantly lengthened (53.7 ± 5.9 days). When females had tactile contacts, the oestrous cycle was further lengthened (62.7 ± 0.8 days). This lengthening of the oestrous cycle was related to an extension of the luteal phase associated with a decrease in progesterone concentrations during this period. In females maintained with one male (paired) or with males and females (heterosexually grouped), large individual variations were shown in cycle lengths or in progesterone concentrations. In these females, cycle lengths and progesterone concentrations were inversely correlated to plasma cortisol concentrations.

**Introduction**

In captivity, as in the wild, the lesser mouse lemur (subfamily Cheirogaleinae) breeds seasonally and changes in reproductive functions are photoperiod-dependent (Petter-Rousseaux, 1980). Under natural photoperiod in Paris, the first seasonal spontaneous ovulation occurs at the end of March and each female experiences 2 or 3 periods of oestrus. The seasonal anoestrus lasts from September to February. In this species, presently considered as representative of the ancestral primate stock (Dutrillaux, 1979), the vagina remains imperforate except during oestrus and at parturition.

The interval between two vaginal openings during the breeding season is highly variable and ranges from 30 to 100 days with a mean length of 50-55 days, whereas gestation lasts 60-62 days (Petter-Rousseaux, 1962; Martin, 1972; Andriantsiferana, 1975; Glatston, 1979; Perret, 1982a). In a previous study (Perret, 1982b), histological investigations revealed that captive lesser mouse lemur females show inhibited gonadotrophic function. Moreover, a high concentration of plasma cortisol was found in females when they were maintained in groups (Perret & Predine, 1984). It was suggested that physio-pathological disturbances (decrease of follicular growth, follicular atresia, cystic or neoplastic formations), and variability in oestrous cycle lengths were due to social influences through an enhancement of corticosteroid secretions.

In the present study, therefore, to provide a more detailed analysis of the ovarian cycle length in the lesser mouse lemur and to investigate the possible role of social interactions, we measured plasma progesterone concentrations in cyclic females in different social living conditions.
Materials and Methods

Animals

The lesser mouse lemurs (*Microcebus murinus*) were laboratory born and were reared from stock originally caught on the southwest coast of Madagascar. The 19 adult females studied were 2–4.5 years old. In captivity, conditions of food availability, temperature and humidity were maintained constant and have been detailed previously (Perret, 1982a). Animals were exposed to the natural photoperiod in Paris 48°N) and the first and second oestrous cycles were studied. To follow social influences, females were maintained in different social environments.

Females without male influences. Totally isolated females (N = 4) were caged individually under conditions of visual and olfactory isolation, although vocal interactions may have existed. Partly isolated females (N = 4) were caged singly but olfactory, visual and vocal communications between them were possible. Females previously caged singly were caged together in a homosexual group (N = 4) after the occurrence of the second oestrous period.

Females with male influences. Each of 3 females was paired with an adult male in separate cages visually isolated from other mouse lemurs. For heterosexually grouped females (N = 8), two dense groups of 4 females with 4 males were kept in large cages.

Blood collections

Blood collections were made by puncture of the saphenous vein throughout 1 year including the non-breeding phase and the following breeding season. Animals were sampled during their daily sleeping period, without prior anaesthesia and blood was collected within 5 min of removing the animal from its nest box. The lesser mouse lemur is a very small primate (body weight about 100 g) and to avoid possible changes in packed cell volume, blood collections were limited. During the non-breeding season, collections were taken every month for all females (200 µl each). During the breeding season, blood samples were taken at weekly intervals during the first 3 months then every 2 weeks (100 µl each) for females without male influences and every 3 weeks (200 µl each) for females with male influences. In these conditions of blood sampling, no significant variation was observed in the packed cell volume. The mean percentage coefficient of haematocrit variation was 5.1 ± 1.6, which is not different from the error of the measurements.

Radioimmunoassay procedures

Progesterone samples were prepared by diluting 40 µl samples of plasma with 960 µl of saline phosphate buffer (0·05 M, pH 7·4, containing 1/1000 gelatin). Tracer amounts of tritiated progesterone were added to each plasma sample before extraction. Progesterone was extracted twice by 5 ml diethyl ether. After centrifugation, the extracts were evaporated to dryness then redissolved in 0·5 ml saline phosphate buffer. After extraction, recovery of progesterone was 77·5 ± 7·5%. Radioimmunoassay was performed on two extracted aliquants corresponding to 4 and 8 µl plasma. The antiserum was raised in a rabbit to \( \Delta^4 \)-pregnen-3,20-dione 11α-hemisuccinate coupled to bovine serum albumin (purchased from Pasteur Institut, Marne la Coquette, France). The percentage cross-reactivities were: progesterone, 100%; deoxycorticosterone, 3%; 6β-hydroxyprogesterone, 1.8%; 5α-pregnan-3,20-dione, 1.6%; other C21 steroids, <1%, and C19 steroids, <0·03%. The intra- and interassay coefficients of variation were 14·9 and 16·1% respectively. The sensitivity of the assay was 5 pg and the minimum detectable concentration in plasma was 1·25 ng/ml.

Progesterone concentrations in plasma measured with or without chromatography on celite columns (Rhoda, Corbier & Roffi, 1984) are significantly correlated (linear regression \( P < 0·001, N = 20 \), showing that chromatographic purification was not necessary.
Cortisol concentrations were measured in plasma of paired and grouped females using radioimmunoassay procedures previously described (Perret & Predine, 1984). The antiserum was raised from 7-carboxymethyl-cortisol coupled to bovine serum albumin; the intra- and interassay coefficients of variations were 11 and 14% respectively. The sensitivity of the assay was 10 pg and the minimum detectable level in plasma was 25 ng/ml.

Vaginal examinations

The sexual state of each female was determined by the appearance of the vulva; observations were made daily during the perioestrous period. Except during oestrus and at parturition, the vagina remains closed. Before the perforation (7–10 days) a pink swelling appears on the vulva and gradually enlarges. During the vaginal opening (4–7 days), ovulation and mating were detected by daily vaginal smears. Spontaneous ovulation occurs 2 or 3 days after the vaginal opening (Perret, 1982a). Brief vaginal perforations without swelling, seen in few females, were not considered as oestrus and vaginal smears only contain mucus and leucocytes.

Statistical analysis

The length of the oestrous cycle was determined as the interval between two true vaginal openings accompanied by a large swelling. Hormonal data are plotted from the first day of the vaginal opening (Day 0). All values are means ± s.d. and statistical differences were tested using variance analysis. To evaluate correlations between specific parameters, we used linear regression analysis.

Results

For 19 females studied during one breeding season, 50 vaginal perforations were observed. Within these 50 oestrous periods, 19 were associated with mating and 14 pregnancies ensued. To define oestrous cycle lengths or progesterone profiles, the last seasonal oestrus (N = 12) for which the cycle length cannot be established was not considered but 25 first or second oestrous cycles were followed.

Oestrous cycle lengths

When females were totally isolated from other conspecifics, the oestrous cycle length was 38.2 ± 5.7 days (N = 4). In females kept singly but able to communicate through olfactory, visual and auditory signals, the interval between two vaginal openings was significantly lengthened: 53.7 ± 5.9 days (N = 4, P < 0.01). When females had tactile contacts with one male or with males and females, this interval was slightly but not significantly lengthened: 56.2 ± 2.0 days (N = 4 cycles) and 58.2 ± 10.9 (N = 9 cycles) respectively. It appeared that the longest interval between 2 periods of oestrus was exhibited in homosexually grouped females: 62.7 ± 0.8 days (N = 4) (Fig. 1).

Progesterone profile during the oestrous cycle

During the sexual rest period (September to January under natural photoperiod in Paris), concentrations of circulating progesterone remained low and in most cases below the sensitivity of the assay, i.e. ≤1.25 ng/ml. From February, progesterone values increased slowly and reached 3.7 ± 1.8 ng/ml (N = 19) in the month before the first occurrence of seasonal oestrus. During the breeding season, progesterone concentrations at oestrus were similar in all females (7.2 ± 5.0 ng/ml) regardless of the social environment or the time of occurrence of oestrus (first,
Fig. 1. Plasma progesterone concentrations during oestrous cycles in lesser mouse lemur females without male influences: (a) totally and (b) partly isolated females, and (c) homosexually grouped females. Progesterone means (± s.d.) are plotted from the first day of vaginal perforation until the following one (Oe). Mean cycle length ± s.d. is indicated in days and stippled areas represent the estimated luteal phase (progesterone ≥ 20 ng/ml).

second or third seasonal oestrus). However, large differences were seen in plasma progesterone profiles during the luteal phase and were related to social environment.

Females without male influences. At 1 week after the first day of vaginal opening, plasma progesterone concentrations in totally isolated females significantly increased from 9.3 ± 4.0 to 22.1 ± 7.0 ng/ml (P < 0.05), remained at 26.0 ± 10.4 by 1 week later and reached an average peak of 108.7 ± 14.1 ng/ml between the 20th and 25th days after oestrus. Then progesterone concentrations abruptly decreased (24.4 ± 17.9 ng/ml) and females entered a second oestrus (Fig. 1a). The length of the luteal phase cannot be accurately defined because of the large interval between two blood samples, but progesterone concentrations > 20 ng/ml may be considered as representative of true luteal activity (value 2-fold higher than that observed at oestrus). The luteal phase, estimated as lasting from the consistent rise of progesterone > 20 ng/ml until levels decreased again below this value, therefore varied between 25 and 30 days.

In partly isolated females, progesterone concentrations increased to 20 ± 5 ng/ml only 2 weeks after the first day of vaginal opening. But, as for totally isolated females, elevated progesterone concentrations occurred (peak: 85.4 ± 17.4 ng/ml) about 30 days after the oestrous period. The subsequent decrease in progesterone concentrations was less marked since high values were seen 45 days after oestrus (46.8 ± 18.1 ng/ml: Fig. 2b), indicating that the luteal phase was lengthened to 35–40 days.

In homosexually grouped females, in which the oestrous cycle lengths were the longest, progesterone concentrations > 20 ng/ml were first measured 2 weeks after vaginal perforation, as for females kept singly. Although progesterone concentrations were highest about 1 month after oestrus, a pronounced peak was absent and values remained about 50 ng/ml (42.5 ± 11.5 ng/ml: Fig. 1c).

Females with male influences. Thirteen oestrous cycles were observed. Since the females were living with a male, these oestrous cycles were those of females that did not mate (N = 8) or of females for which mating was not successful (N = 5). Not all females living with males became
pregnant. In pairs or in groups, females almost always outranked males in fights and some of them, mostly heavy females, did not accept mounts and copulations. It was difficult to determine a typical progesterone profile in females with male influences owing to the large individual variations. Nevertheless three different progesterone profiles could be distinguished (Fig. 2). In 3 cycles, progesterone concentrations increased significantly midway between 2 oestrous periods (25–35 days after oestrus) and the progesterone peak was comparable to that observed in partly isolated females (> 60 ng/ml). In 7 cycles, an increase in progesterone concentrations was observed between the 20th and the 35th day after the first day of vaginal perforation. The rise was clearly distinguishable but always below 50 ng/ml. In the 3 remaining cycles, the progesterone concentrations remained below 20 ng/ml throughout the period between 2 vaginal perforations. This could perhaps correspond to an anovulatory cycle because although these females exhibited a normal vaginal smear cycle and copulated, they did not conceive. There was a significant inverse correlation ($P < 0.05$) between the oestrous cycle length and the values in progesterone concentrations recorded about 1 month after oestrus. Generally, during the breeding season, a female exhibited the same progesterone profile for two successive oestrous cycles. Cycles with progesterone values of <20 ng/ml were only found in females living in dense social grouping. The other cycles were equally distributed in paired or grouped females.

**Interrelations with cortisol**

Plasma cortisol concentrations were measured in paired and heterosexually grouped females. Related to the three different progesterone profiles described for their oestrous cycles, important variations in plasma cortisol concentrations were recorded. During the oestrous period, mean cortisol concentration was 160 ± 75 ng/ml for females which exhibited an oestrous cycle with a pronounced progesterone increase; it significantly increased when progesterone concentrations recorded midway between two periods of oestrus decreased (Fig. 3). These differences in mean cortisol concentrations were maintained during the period between two vaginal openings since

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**Fig. 2.** Plasma progesterone concentrations during oestrous cycles in lesser mouse lemur females with male influences. Three progesterone profiles were distinguished: □, with a high progesterone peak (N = 3); ●, with a moderate progesterone increase (N = 7); and ▲ without progesterone increase (N = 3). Individual data are plotted from the first day of vaginal perforation and arrows indicate the following oestrus (Oe) for the three different progesterone profiles.
cortisol increased slightly (but not significantly) in all females after oestrus. It appeared that a female which exhibited high cortisol concentrations at oestrus had a longer cycle ($P < 0.05$) and less elevated progesterone concentrations after oestrus ($P < 0.01$). Theoretical characteristics of the oestrous cycle in lesser mouse lemurs may be extrapolated from the regression lines of these significant correlations: cycle length of 45 days, progesterone levels peaking at 104 ng/ml. These theoretical values are closely similar to those found in totally isolated females.

**Discussion**

In the lesser mouse lemur, the inter-oestrus period has been described as lasting 38 to 100 days (50–60 days on average) during the photoperiodically regulated breeding season (see ‘Introduction’). In the present study, when oestrous cycles of females maintained in different social environments were compared, it was found that the inter-oestrus period depended mainly on social communication between females: it lasted about 40 days when females were totally isolated but was lengthened when females could communicate through visual and olfactory signals, and was still longer when females had tactile contacts. Reproductive inhibition between female rodents has been well documented and involves urinary pheromonal signals (see review by Marchlewsk-Koj, 1984). The role of olfaction on reproductive biology has been stressed in mouse lemurs which possess a well developed olfactory system and vomeronasal organ, and which exhibit numerous behavioural patterns associated with olfactory signals (Schilling, 1979). In male mouse lemurs, sexual inhibition can be induced by exposing isolated males to the urinary odour of a dominant conspecific (Schilling, Perret & Predine, 1984). A similar olfactory mechanism may cause the lengthening of oestrous cycle in the female. As in grouped female mice (Reynolds & Keverne, 1979), the vomeronasal organ could be involved in mouse lemurs since the lengthening effect was more pronounced when tactile contacts between females were possible.

Within heterosexually grouped females, the variability in oestrous cycle lengths may be

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**Fig. 3.** Mean ± s.d. plasma cortisol concentrations in lesser mouse lemur females living with males according to the values of progesterone recorded at about 1 month after oestrus: values at oestrus □ and during the luteal phase □. The numbers of females are indicated. *$P < 0.05$.**

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explained by two conflicting influences: the inhibitory effect of females and the stimulatory effect of males. In rodents, the influence of male contacts on female reproductive function is mainly a stimulatory effect which also involves chemical cues (see Marchlewksa-Koj, 1984). In mouse lemurs, the role of the male upon the oestrous cycle remains unclear. Nevertheless, it has been observed in a previous work that the first seasonal oestrus occurred earlier in groups in which more than 3 males were present, i.e. when male dominance was strong (Perret, 1985).

In social primates, numerous reports have dealt with socially induced reproductive suppression in subordinate females (Bowman, Dilley & Keverne, 1978; Abbott, 1984; Epple & Katz, 1984), but amongst grouped female mouse lemurs, no clear rank order appears because of the lack or the variability of aggressive interactions between them. Consequently, it was not actually possible to interpret the difference in cycle lengths as proceeding from dominance-subordination relationships even though they may exist.

In the wild during the breeding season, mouse lemurs are encountered singly when active at night but may communicate through visual, auditory and especially olfactory signals (Martin, 1972). Partial isolation would theoretically be a more appropriate situation to define normal reproductive conditions in captivity. However, the density of social signals existing even between females that are partly isolated is greatly exaggerated compared to the natural situation. Therefore, I assume that the oestrous cycle recorded in totally isolated females is closer to the natural reproductive cycle. In these females, the oestrous cycle length is about 40 days which is very similar to the cycle length recorded in prosimians from hormonal evaluations: 40 days in Lemur variegatus and L. catta, 33 days in L. macaco and 44 days in Galago crassicaudatus (Eaton, Slob & Resko, 1973; Van Horn & Resko, 1977; Bogart, Kumamoto & Lasley, 1977). In contrast, the oestrous cycle length in mouse lemurs differs from those recorded by vaginal or behavioural examinations in Cheirogaleus medius (19 days) and C. major and Microcebus coquereli (30 days) (Petter-Rousseaux, 1962, 1980; Foerg, 1982) and seems closer to those of Iorisids (Petter-Rousseaux, 1962; Vincent, 1968; Darney & Franklin, 1982; Izard & Rasmussen, 1985).

The reproductive cycle of lesser mouse lemurs includes a period of 10-15 days which could be considered as the follicular phase as in menstrual cycles among higher primates. During this period when progesterone levels were low, changes in external genitalia take place with a gradual swelling of the vulva reaching a maximal development just before the vaginal perforation. These changes in the reproductive tract and the induction of behavioural oestrus would indicate an increasing oestrogen secretion as described in many primates (Reynolds & Van Horn, 1977). The duration of the 'follicular phase' may also be appreciated from the recurrence of oestrus after parturition: a vaginal oestrus with large swelling was observed 15-20 days after delivery and earlier (10.2 ± 2 days, N = 5) when the young were born dead or did not survive because of a lactation insufficiency (M. Perret, unpublished data). Spontaneous ovulation occurs 2 or 3 days after the vaginal perforation (Perret, 1982a) and oestrus does not last longer than 1 day. The sexual receptivity of the female is restricted to the periovulatory period (2-4 h according to Lebec, 1984). The length of the luteal phase was estimated to be 20-30 days. It compares well with data from other prosimians: 24 days in G. crassicaudatus or 24-28 days in Lemur (Eaton et al., 1973; Bogart et al., 1977). Progesterone concentrations reached high levels (∼100 ng/ml) as in L. catta (Van Horn & Resko, 1977) or to a lesser extent in L. variegatus (Bogart et al., 1977).

Since sexual receptivity is consistently limited to the ovulatory period as in a true oestrous cycle, and the extended periovulatory period can be compared to the follicular phase in a menstrual species, the ovarian cycle of the lesser mouse lemur may represent an intermediate stage in evolutionary trends of mammalian reproduction.

In rhesus monkeys, an inadequate luteal phase (progesterone output lower than expected) is associated with a decrease in FSH:LH ratio in the preceding follicular phase (Wilks, Hodgson & Ross, 1976; Goodman & Hodgson, 1983). Similarly, inadequate LH or oestrogen secretions have been described for inhibited cycles of subordinate females (Abbott, 1984). In the mouse lemur, inappropriate secretion of gonadotrophin hormones would lead to reduced follicular development...
and abnormal luteinization, and the maintained, but less active, corpus luteum would inhibit the growth of a new follicle and so delay a further cycle.

In many rodents, decreasing gonadotrophin secretions under social pressure have been linked to a stimulation of the hypothalamoadrenal axis (see review by Andrews, 1979) and such relationships have also been described for primates. In rhesus monkeys, ACTH blocks the preovulatory LH surge and lengthens ovarian cycles (Moberg, Watson & Hayashi, 1982) and decreased LH secretion is related to high corticoid concentrations in women (Thiebaut, Luton, Mahoudeau & Bricaire, 1979; Vierhapper, Waldhauss & Nowotny, 1981). In lesser mouse lemurs activation of the cortico-adrenal axis seems to be implicated since an inverse correlation was found between plasma cortisol concentrations and cycle lengths, and cortisol and progesterone concentrations during the luteal phase. The generally accepted role for glucocorticoids in reproductive functions is to decrease the secretion of FSH and LH. Such a fall in gonadotrophin output in lesser mouse lemurs is supported by histological examination of the ovaries of captive females revealing many atretic follicles (Perret, 1982b), and by undetectable levels of oestrogens in this species (Glatston, 1979), which would be indicative of reduced follicular development.

Nevertheless, an ovarian inhibition of FSH and LH by prolactin mediation has been suggested (Keverne & de la Riva, 1982) and Aso et al. (1982) have demonstrated that the baboon corpus luteum derived from a follicle exposed to elevated concentrations of prolactin during its maturation subsequently exhibits insufficient function. It would be of interest to test the involvement of prolactin in female mouse lemurs in which ovarian cycles can be strongly modified by social factors.

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