Reproductive Biology of Female Titi Monkeys

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Titi monkeys (Callicebus spp., Cebidae) are monogamous neotropical primates that live in family-like groups typically consisting of an adult monogamous pair and one or two young. Knowledge about the reproductive biology of this genus is scanty. This study investigated the reproductive biology of female dusky titi monkeys (Callicebus moloch). An initial analysis characterized reproductive parameters of 32 females from a captive colony maintained for 23 years at the California Regional Primate Research Center (CRPRC). The colony records provided data on reproductive parameters such as interbirth intervals, seasonality, age at first pregnancy, and reproductive rate in captivity. Changes in urinary levels of estrone conjugates (E1C) and pregnanediol-3alpha-glucuronide (PdG) were used to characterize major reproductive events. Urine samples from eleven females were collected during 17 months. The endocrine data were used to examine changes associated with cycling, conception, and the post-partum period as well as to determine the duration of the ovarian cycle and gestation length. The analysis of colony records indicated that females whose infant survived through weaning gave birth at intervals remarkably close to one year, while those who lost their offspring showed a significantly shorter interval. As long as they lived within the family group, mature female offspring did not breed. The analysis of the endocrine profiles indicated that after giving birth to a viable offspring, females undergo a relatively prolonged period of anovulation (approx. 6.5 months), followed by 1-3 non-conceptive cycles (approx. 1 month), after which they conceive and gestate (4.3 months). Am. J. Primatol. 47:183–195, 1999. © 1999 Wiley-Liss, Inc.

Key words: female reproductive biology, Callicebus, neotropical primates, urinary hormones

INTRODUCTION

Titi monkeys (Callicebus spp.) are small neotropical monkeys that inhabit most of the tropical forests in South America. The few field studies conducted on

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Received for publication 11 February 1998; revision accepted 28 August 1998

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titi monkeys have focused on various aspects of their social organization and behavioral ecology [Mason, 1966; Robinson, 1979, 1981; Wright, 1985; Müller, 1996; Kinzey, 1997]. Field observations suggested that they live in family-like groups typically consisting of an adult monogamous pair and one or two young. Since the groups normally include only one infant, it has been assumed that only one female in the group breeds. The reproductive female gives birth to singletons which presumably disperse from the natal group when they reach sexual maturity. Given that titi monkey females do not menstruate or show changes in the external genitalia that could be observed in the field, it has not been feasible to examine how their social organization and behavior correlate with the females’ reproductive status. Therefore, many questions remain unanswered regarding the mechanisms that contribute to shape the reproductive biology of this genus.

Laboratory studies have broadened our knowledge of titi monkeys’ social organization by providing some insights into the behavioral mechanisms underlying their mating and parenting behavior. Studies of the dusky titi monkey [Callicebus moloch, but C. cupreus following Hershkovitz, 1990] have shown that male-female pairs form a strong bond [Cubicciotti & Mason, 1975, 1978; Mason, 1974; 1975; Mendoza & Mason, 1986a; Anzenberger, 1993; Fernandez-Duque et al., 1997]. Adult males participate actively in the care of their offspring from the first few days of the infant’s life [Fragaszy et al., 1982; Mendoza & Mason, 1986b], while siblings are rarely involved in infant care [Jantschke, 1992].

The physiological mechanisms underlying titi monkey mating and parenting behavior have received less attention. To date, there has been only one preliminary study that examined selected aspects of the breeding biology of captive titi monkeys [Jantschke et al., 1996]. This study investigated salient aspects of the reproductive biology of female titi monkeys. Initial analysis utilized actuarial data contained in records of the colony maintained at the California Regional Primate Research Center (CRPRC) for the past 23 years. From these data it was possible to evaluate reproductive parameters such as interbirth intervals, seasonality, age at first pregnancy, and reproductive rate in captivity. Changes in urinary steroid metabolites from a subset of females were used to characterize reproductive events, including the ovarian cycle, first days of gestation, and the post-partum period.

METHODS

Colony records

Fifty adult Callicebus moloch females were maintained at the CRPRC between April 1972 and August 1995. Eighteen females had no live offspring. Of the remaining 32 females, 23 were laboratory born and 9 were wild-caught and imported as adults. Each individual female’s record contained life-history information on reproductive history, parentage (if known), and all changes in housing conditions including addition or removal of cagemates as well as cage moves. The records of the 32 females covered 1 to 15 years during which each female had 1 to 11 offspring.

Subjects and Housing

Subjects remained with their parents in family groups until they were at least one year of age. After this time, females were paired with an adult male as
soon as caging and a suitable mate were available. Mean age at first pairing for laboratory-born animals was $2.1 \pm 0.8$ years (mean $\pm$ SD, range = 0.9–3.6, n = 23). Whenever one member of the pair died, a new mate was provided as soon as possible. The mean length of time elapsed from death of a partner to provision of a new one was $36.8 \pm 52.2$ days (range = 1–130, n = 12).

Aside from cage changes required by pairing or changes in cage type, animals were only removed from their home cages for experimental or clinical reasons. Brief separations (1–2 hr) occurred regularly for experimental purposes. Experimental or clinical procedures resulted in 40 overnight separations of 1 to 42 days with a mean of $11.2 \pm 10.3$ days in the 23-year period.

Pairs and their offspring were housed in indoor, stainless steel cages ($1.2 \times 1.2 \times 2.1$ m) containing 3 perches extending the width of the cage and arranged in a step-wise fashion. Opaque sides and back of cages minimized visual interaction between adjacent groups; fronts, tops and bottoms of cages were wire mesh. Skylights in the ceilings of the building exposed the subjects to sunlight and natural variation in day length. Forced air heating and cooling maintained room temperature at 22–33°C. Sliding garage doors could be opened when the daytime ambient temperature permitted. Supplemental lighting was provided 12 h per day with lights on at 0600 hr. For caging arrangements prior to 1990 see Mendoza & Mason, 1986a and Menzel, 1986.

In all housing arrangements, monkeys were maintained according to standard laboratory protocol, which included twice daily feeding at approximately 0800 and 1400 hr. Although specific dietary constituents varied, animals were always fed a combination of commercially available monkey food, protein supplements, and fresh fruits and vegetables. Water was available ad libitum.

Eleven adult females were selected for endocrine analyses. Eight females were laboratory-born and were raised in family units, three females were wild-born and imported as adults. The laboratory-born females ranged in age from 2.5 to 15.3 years (mean $\pm$ SD = 7.0 $\pm$ 4.7 years) at the beginning of the study. Subjects had been housed with an adult male for at least 6 months before data collection began. All females conceived or gave birth at least once during the study.

Sample collection and analysis

Urine samples were collected during the first morning void (0530–0600 hr), four to six times a week, with a maximal interval of three days between collection of successive samples for any given individual. Samples were collected for 17 months between March 1992 and August 1993. Within 30 min of collection, samples were frozen and stored in plastic polypropylene vials at $-20$°C until assayed.

Urine samples were assayed via enzyme-immunoassays (EIAs) previously characterized for estrone-1-conjugates ($E_1C$) and pregnanediol-3-glucuronide (PdG) [Shideler et al., 1990, Munro et al., 1991]. Urinary creatinine (Cr) concentrations were estimated using Taussky’s method [Taussky, 1954]. All hormone concentrations are expressed as ng of hormone per mg of Cr.

Intra- and inter-assay coefficients of variation for the $E_1C$ EIA averaged 7.34% and 9.94%, respectively. For the PdG EIA, intra- and inter-assay coefficients of variation averaged 7.72% and 13.23%, respectively. Comparison of urinary $E_1C$ and PdG with plasma levels of estradiol and progesterone indicated a high correlation between plasma hormones and next day urinary metabolites for each female (Valeggia, 1996).
Definitions and criteria for characterization of ovarian activity

Ovarian cycles. We utilized the criterion of progesterone-defined ovulation to identify ovulatory cycles. Changes in PdG levels of 400 ng/mg Cr or more were considered to be evidence that the cycle was ovulatory. There were few instances of hormonal profiles exhibiting evidence of intermediate progesterone activity. PdG levels were either consistently low (below 80 ng/mg Cr) or exhibited a clear peak of at least 600 ng/mg Cr within a 14 day period.

The following measures were used to describe each cycle: concentration of E₁C at the preovulatory (follicular) and postovulatory (luteal) peaks, concentration of PdG at the ovulatory peak, concentration of both metabolites at the intercycle troughs, and number of days between intercycle troughs and between E₁C and PdG peaks.

Conceptions and gestation length. A conception was identified when the concentration of urinary PdG and E₁C following cycle peaks remained elevated (i.e. failed to return to average intercycle trough levels) for at least 13 days after the PdG peak. The 13-day criterion was chosen based on the maximum estimate for a non-conception follicular phase of ten days plus three days to account for the maximal interval between samples during sample collection. Samples corresponding to day 30 after conception and beyond were not analyzed, since the focus of this study was not the endocrine characterization of pregnancy per se.

For estimating gestation length, we considered the day of ovulation to be the day before the urinary PdG peak (progesterone-defined ovulation). Thus, we calculated gestation length as the number of days from the progesterone-defined ovulation day to the day of birth of a full-term live infant. An infant was considered to be full-term when its birth weight fell within the range typical for the colony and it survived for at least seven days.

Post-partum period. Following birth, urinary hormones were examined for evidence of post-partum estrus. Only females whose infants survived until the next conception were included in the analysis.

Statistical analyses

The effect of age of the female on time from pairing to a live birth was tested using a regression analysis. Differences between interbirth intervals for females who lost their infants versus females whose infant survived were evaluated using t-tests. Seasonality was examined by evaluating departures from a random birth frequency distribution across calendar months using Chi-square tests. Statistical tests used a level of significance of 0.05.

RESULTS

Analysis of colony records

From the records of the 32 females, we obtained information on number of births, rate of twinning, age at first live birth, birth seasonality, birth rate, time to birth after pair formation, interbirth intervals, gestation length, and reproductive history of mature daughters living with their parents.

A total of 148 births were recorded. The incidence of twinning was low (2 births, 1.4%); in both cases one of the infants died on the same day of birth. No instances of births, stillbirths, or abortions were recorded from female daughters living exclusively with their parents. Although one female gave birth to a live infant while housed with her father and his mate in an outdoor enclosure (Menzel,
1986), males from neighboring groups could have had sexual access to this female and, therefore, paternity by males other than the father cannot be ruled out. For experimental reasons, a group of 11 female offspring were still housed with their parents in August 1995, and, although undoubtedly at reproductive age (2.5–3.6 years of age), none of these females conceived while living with their parents.

For females housed with an unfamiliar male, the mean age at first live birth was 3.7 ± 1.3 years (range = 2.0–6.9, n = 23, only laboratory-born females considered). It must be noted, however, that the age at first parturition was dependent, in large part, by the age at pairing.

Females gave birth approximately one year after being paired with an unrelated adult male. Mean time from formation of a reproductive pair to a live birth was 13.4 ± 8.7 months (range = 4.2–56.8, n = 41 pairings, 32 females). Age of the mother had a negligible effect on time from pairing to live birth as suggested by regression analysis (r = 0.12, df = 29, n = 41 pairings, p = 0.33). However, parenting experience seemed to have an important effect on time to live birth after repairing. Females who had had an offspring with a previous mate showed an average time from re-pairing to live birth of 10.8 ± 6.8 months (range = 4.2–23.4, n = 22 females). For nulliparous females, the average time from pairing to live birth was 18.5 ± 13.1 (range = 4.6–56.8, n = 19) months. This difference was statistically significant (t = 2.58, df = 39, p = 0.016).

Records from 27 females (20 laboratory-born, 7 wild-born) who had had at least two full-term births were selected for the analysis of interbirth interval data. One hundred and seventy-two births provided data on 145 interbirth intervals. Interbirth interval data for each female were averaged and then an overall average computed across females. The average interbirth interval was 335.5 ± 11.2 days (range = 128–687, n=27 females). The duration of the interbirth interval was influenced by the viability of the offspring. Females whose infants survived until the subsequent birth showed a mean interbirth interval of 363.6 ± 12.5 days (range = 248–687, n = 23 females). Females who lost their infants within one week of birth gave birth an average of 249.6 ± 13.0 days after the loss (range = 128-422, n = 20 females). This difference is statistically significant (t = 10.06, df = 41, p < 0.001).

Females gave birth at all times of the year (Fig. 1), although there was a clear birth peak during the winter months. Sixty-percent of births occurred between December and March (χ²(11)= 52.25, p < 0.001).

Ovarian cycles

Twelve cycles from six females were analyzed. Four females contributed two cycles each; the other two females contributed one and three cycles each. Titi monkey females’ ovarian cycles have two distinct phases (Fig. 2): an early one characterized by a peak in levels of E₁C (follicular phase), and a second phase in which both E₁C and PdG levels are elevated (luteal phase). Cycle length, calculated as number of days between E₁C intercycle troughs averaged 17.2 days (± 1.5), whereas the cycle averaged 17.7 days (± 1.2) when number of days between PdG intercycle troughs was considered. E₁C and PdG mean values for the ovarian cycle are presented in Table I. These data were used to plot the endocrine profile shown in Fig. 3.

Conception and gestation length

Fifteen conceptions from ten females were analyzed. Five females contributed two conceptions each; the other five females contributed one conception
The follicular phase and postovulatory peak values did not differ significantly from the values obtained for the non-conceptive ovarian cycles (t-tests, all p's > 0.05).

Figure 4 illustrates the hormone profile of an adult female in the period surrounding conception. On average, after the ovulatory PdG peak, E1C levels remained high (mean ± SD; 1050.6 ± 50.9 ng/mg Cr) for 6.4 ± 0.3 days, and then increased again around day 21 reaching a plateau corresponding to the first days of pregnancy. Urinary PdG levels declined after the ovulatory peak for an aver-
age of 3.1 ± 0.7 days and then showed a second peak. This second increase occurred, on average, 5.0 ± 0.9 days after the ovulatory PdG peak. Urinary concentration of PdG continued to increase thereafter until day 20 when a plateau was reached. These data and the values in Table I were used to plot a profile for conceptive cycles (Fig. 5).

Eight pregnancies from seven females were considered for the estimation of

Table I. Mean Values (± SD) of Urinary E1C and PdG Characteristic of Ovarian Cycles, Conception, and Post-Partum Period

<table>
<thead>
<tr>
<th>Period</th>
<th>Number of cycles</th>
<th>Number of females</th>
<th>E1C (ng/mg Cr)</th>
<th>PdG (ng/mg Cr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian cycles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercycle troughs</td>
<td>12</td>
<td>6</td>
<td>222.2 ± 33.1</td>
<td>74.2 ± 14.3</td>
</tr>
<tr>
<td>Preovulatory peak</td>
<td>12</td>
<td>6</td>
<td>998.9 ± 30.7</td>
<td></td>
</tr>
<tr>
<td>Postovulatory peak</td>
<td>12</td>
<td>6</td>
<td>1,208.3 ± 37.5</td>
<td>801.1 ± 39.7</td>
</tr>
<tr>
<td>Conceptions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercycle troughs</td>
<td>15</td>
<td>10</td>
<td>286.2 ± 82.8</td>
<td>82.6 ± 25.9</td>
</tr>
<tr>
<td>Preovulatory peak</td>
<td>15</td>
<td>10</td>
<td>1,020.5 ± 42.9</td>
<td></td>
</tr>
<tr>
<td>Postovulatory peak</td>
<td>15</td>
<td>10</td>
<td>1,124 ± 42.3</td>
<td>799.2 ± 33.5</td>
</tr>
<tr>
<td>Pregnancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First days of pregnancy</td>
<td>15</td>
<td>10</td>
<td>1,869.4 ± 55.6</td>
<td>1,423.5 ± 71.7</td>
</tr>
<tr>
<td>Last five days of pregnancy</td>
<td>14</td>
<td>9</td>
<td>4,290.3 ± 282.2</td>
<td>1,345 ± 402.8</td>
</tr>
<tr>
<td>Post-partum period</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First day post-partum</td>
<td>14</td>
<td>9</td>
<td>343.6 ± 46.1</td>
<td>117.6 ± 16.3</td>
</tr>
<tr>
<td>Overall post-partum*</td>
<td>14</td>
<td>9</td>
<td>75.9 ± 15.0</td>
<td>14.0 ± 8.6</td>
</tr>
</tbody>
</table>

*Values in this row represent mean levels from day 2 post-partum to the day before the beginning of first post-partum ovulatory cycle.
gestation length. Taken as the number of days from the progesterone-defined ovulation day to the day of birth, the average gestation length was 128.6 ± 4.4 days (range 122–135).

Post-partum period

Fourteen post-partum periods from nine females were analyzed. Five females contributed two periods each; the other four females contributed one period each. E1C and PdG mean values during the last 5 days of pregnancy and the day after birth are presented in Table I. The urinary concentration of gonadal hormones remained low and acyclical until the next ovulatory cycle (Overall postpartum, Table I). This period of anovulation lasted, on average, 194.1 ± 25.1 days.

On average, females conceived 31.3 ± 9.2 days after resuming ovarian activity following parturition. Seven of the fourteen conceptions occurred on the first cycle after parturition, four occurred on the second, and three on the third post-partum cycle. For females with infants, the average time elapsed from birth to the next conception was 225.4 ± 38.7 days.

The profile of urinary hormone excretion for an adult female is shown in Fig.6. The figure includes the last days of pregnancy, birth of a live offspring, lactational anovulation, resumption of cycling, conception, and early pregnancy, spanning 275 days of sample collection.

DISCUSSION

This study provides the first detailed account of female titi monkey’s reproductive biology based on an analysis of reproductive events in a captive colony.
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Fig. 5. Conceptive cycle based on average hormonal values from 9 conceptions (n = 6 females). (1) preovulatory E1C peak, (2) postovulatory E1C peak, and (3) ovulatory PdG.

as well as on patterns of urinary gonadal hormone excretion. Our analyses of colony records show that *Callicebus moloch* reproduces well in captivity as indicated by 148 births recorded during the 23 years that the colony has existed. Only 1.4% of births were twins, in general agreement with what seems to be the norm for haplorrhine primates [Dunbar, 1995].

Females who lose an infant at birth or soon after, conceive again promptly,

Fig. 6. Urinary hormone excretion profile of an adult female: Birth (day 5); Lactational anovulation period (~ days 6 to 200); First post-partum cycle (~ days 205 to 220); and Conception (~ day 250).
regardless of time of the year. Loss of an infant is followed by a rapid onset of hormonal activity, as suggested by shorter interbirth intervals following stillbirths or neonatal deaths. This suggests that the post-partum period of anovulation does not reflect a truly seasonal ovarian quiescence, but rather the contraceptive effects of lactation. The combination of three reproductive phases—gestation, anovulation, and cycling to conception—yields an interbirth interval remarkably close to one year, giving the impression that the distribution of births may be seasonal and, in our colony, concentrated around the winter months.

Females in the colony reproduced only when paired with an unfamiliar male. As long as they live within the family group, mature female offspring do not breed. It could be argued that no female offspring became pregnant while in their natal group because they were removed before achieving reproductive maturity. However, none of the mature females who were still living with their parents when they were between 2.5 and 3.6 years of age became pregnant or showed signs of sexual/reproductive activity. Considering that females as young as two years can conceive and give birth to viable offspring, this could indicate a case of social suppression of reproduction similar to the one described for callitrichid species [Abbott, 1991].

Females gave birth approximately a year after being paired with a new mate. Parenting experience had a major effect in determining the speed with which females resume reproduction after being paired with a new mate, regardless of age of the female. Nulliparous females took almost twice as long to give birth after being paired with a new mate than multiparous females. At present, we do not have an explanation for this finding. We are currently exploring the possibility that reversal of reproductive suppression requires more time in *Callicebus* than has been reported for callitrichid species. It is also possible that prior fathering experience of the males contribute significantly to infant survival (Mendoza and Mason, 1986b).

Analyses of endocrine data revealed that, after giving birth to a viable offspring, females undergo a relatively prolonged period of post-partum anovulation (approx. 6.5 months), followed by 1–3 non-conceptive cycles (approx. 1 month), after which they conceive and gestate (4.3 months) as indicated by the endocrine profiles. The post-partum period is characterized by low, acyclic levels of reproductive hormones. This period is relatively long; more than half of the interbirth interval is spent in ovarian quiescence. On the other hand, the time to conception after resumption of ovarian activity is relatively short. Typically, titi monkey females conceive in their first or second post-partum cycle, indicating these cycles are indeed ovulatory. Estimates of gestation length from endocrine data and previously published colony records from another laboratory [Jantschke et al., 1996] point to a gestation period of approximately 128 days.

The ovarian cycle of titi monkey females lasts approximately 17 days. Follicular and luteal phases, marked by well-defined E$_1$C and PdG peaks, can be discerned in the endocrine profiles. Differences in the late luteal phase profiles are evident when non-conceptive and conception ovarian cycles are compared. Urinary E$_1$C concentration shows a sustained increase following the post-ovulatory peak. Conceptions are also readily identifiable from visual examination of the profile. The second peak in urinary PdG levels may indicate the recovery of the corpus luteum after conception.
In a comparative perspective, the reproductive characteristics of female titi monkeys conform to the general pattern described for other cebid species. In particular, many aspects of the reproductive biology of *Callicebus* are strikingly similar to the ones reported for owl monkey females (*Aotus spp.*). In nature, they both live in family-like groups consisting of a monogamously mated pair and younger animals [Wright, 1981]. Females in both genera give birth to singletons once a year, following an almost identical gestation period (133 days for *Aotus*, Hunter et al., 1979). Like *Callicebus*, captive owl monkey females, give birth at all times of the year and exhibit a lengthy post-partum period of infertility [Dixson, 1994]. The length of the ovarian cycle (13-19 days) and the typical profile of reproductive hormones are similar in the two species (for *Aotus*, see Dixson, 1994). This information may offer some insights to the current debate on the phylogenetic relatedness of these two genera [Schneider & Rosenberger, 1996].

In summary, the primary contribution of these two studies is the characterization of the reproductive biology of captive *Callicebus moloch* females, including the reliable detection of ovarian function, conception, and post-partum anovulation. It is hoped that the information provided here will be applicable to the understanding of the reproductive characteristics of other *Callicebus* species, as well as to a broader understanding of the reproductive biology of other New World primates.

**CONCLUSIONS**

1. The combination of gestation (~ 4.3 months), lactational anovulation (~ 6.5 months), and time-to-conceive (~ 1 month) results in an interbirth interval remarkably close to one year.
2. Interbirth intervals were significantly shorter for females who lost their infants than for females whose infant survived.
3. Females in the colony reproduced only when paired with an unfamiliar male.
4. Female titi monkeys typically conceived in their first or second post-partum ovulation.
5. The length of the ovarian cycle is approximately 17.5 days, while gestation typically lasts 128 days.
6. The appearance of reproductive seasonality in captive titi monkeys can be attributed to endocrine constraints imposed by pregnancy, lactation, and time to conception.

**ACKNOWLEDGMENTS**

We thank E. Lowe and 16 undergraduate students for their invaluable help with urine collection. This research was supported by grant RR00169 from the National Institutes of Health, United States Public Health Service. CRV was partially supported by fellowships from the Wildlife Management and Conservation Training Program of the National Zoological Park, the American Association of University Women, P.E.O and Organization of American States during part of the study. EFD was partially supported by fellowships from the Wildlife Management and Conservation Training Program of the National Zoological Park, and the Organization of American States. The authors thank four anonymous reviewers for their constructive and helpful suggestions.
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