



Endocrine Monitoring of Wild Dominant and Subordinate Female *Leontopithecus rosalia*

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*In captive callitrichid primates, female reproductive function tends to vary with social status. However, little is known about the interplay between these factors in wild groups. We report observations on normative ovarian function in dominant and subordinate female golden lion tamarins (*Leontopithecus rosalia*) living in wild groups. We monitored ovarian status by measuring, via enzyme immunoassay, concentrations of excreted pregnanediol glucuronide (PdG) and estrone conjugates (E_1C) in fecal samples collected noninvasively from individuals in social groups in the Poço das Antas Biological Reserve, Rio de Janeiro State, Brazil. Dominant breeding females demonstrated steroid levels similar to those previously reported for wild cotton-top tamarin females, with statistically significant rises during pregnancy. The duration of elevation of fecal steroids in breeding females was ca. 4 mo, which corresponds with estimates of gestation from captive studies. Low steroid concentrations from December to June suggest a seasonally-related period of infertility in female golden lion tamarins. Dominant and subordinate females demonstrated several differences in endocrine function. In general, younger females living in intact natal family groups showed no evidence of ovarian cyclicity. We noted endocrine profiles consistent with ovulation and subsequent pregnancy for behaviorally subordinate females living in groups with unrelated males or in which a reversal in female dominance status occurred. Results suggest that in addition to changes in female reproductive endocrinology associated with*

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puberty, the regulation of reproduction in females in wild callitrichid groups can be sensitive to status and relatedness to breeding males.

KEY WORDS: ovarian cycles, fecal steroids, seasonality, reproductive suppression.

INTRODUCTION

In groups of cooperatively breeding mammals, reproduction is typically limited to a single, dominant female (Creel and Waser, 1997; French, 1997; Moehlman and Hofer, 1997). In the callitrichid primates, a variety of mechanisms can produce this dramatic reproductive skew among females. The mechanisms include suppression of ovulation and ovarian production of steroid hormones in older subordinates (Abbott and George, 1991; Carlson *et al.*, 1997; French *et al.*, 1984; Küderling *et al.*, 1995; Saltzman *et al.*, 1997a; Savage *et al.*, 1988; Smith *et al.*, 1997) and behaviorally-mediated mechanisms such as agonistic interactions or inhibition of sexual behavior (incest avoidance; French and Inglett, 1989, 1991; Saltzman *et al.*, 1997b; Ziegler and Sousa, 2002). Most studies of the proximate mechanisms underlying reproductive skew in callitrichid social groups have been conducted under captive conditions. It seems likely that the rules regulating reproductive function in subordinates might operate differently under field conditions, where energy constraints, e.g., foraging and food acquisition, travel, and predator avoidance, are more likely to shape reproductive decision-making. Under these conditions, resources to support reproduction by dominant breeders might be limited, or reproductive attempts by subordinates may be more risky than under more benign captive conditions. In either event, we might expect to see more dramatic socially-mediated reproductive inhibition in wild than in captive callitrichid primates. Conversely, without the constraint of enforced proximity to dominant females that cage-housing produces, subordinate females may be less likely to encounter potential inhibitory stimuli emanating from dominant females, at least during active phases of the day, and may also be more likely to encounter stimulatory cues from unrelated males that are dispersing or in adjacent groups (Digby, 1999; Digby and Ferrari, 1994; Lazaro-Perea, 2001). Under these conditions, then, we might expect less pronounced inhibition among subordinate females.

The development of noninvasive hormone monitoring methodologies (Whitten *et al.*, 1998) has allowed reproductive biologists to address questions regarding endocrine mechanisms of reproduction in contexts in which complex ecological and demographic factors have full play. Indeed, some important differences in the regulation of reproduction in callitrichid primates have been revealed in preliminary studies on cotton-top tamarins (*Saguinus oedipus*). In captive settings, daughters and subordinate females in social

groups of cotton-top tamarins are universally anovulatory while in an intact family group (French *et al.*, 1984; Savage *et al.*, 1988; Ziegler *et al.*, 1987). However, a recent study on a single group of wild tamarins indicated that all daughters in the group ($n = 3$) showed signs of ovarian activity as reflected in periodic elevations in fecal progesterone and estrogen metabolites (Savage *et al.*, 1997). These results confirm our suspicion that the regulation of reproduction in females in wild callitrichid groups may be more complicated than we would expect based on results from captive animals.

In captive golden lion tamarins, details of ovarian function in adult females have been well-established via noninvasively collected urine samples. Nonconceptive ovarian cycle length is 19.6 d, and endocrine profiles suggest a gestation length of 125 d (French *et al.*, 2002; French and Stribley 1985). In contrast to most other callitrichid primates, subordinate females and daughters in captivity exhibit no social suppression of ovarian function, as assessed by excreted urinary steroids, past the age of puberty (~ 14 – 16 mo; Chaoui and Hasler-Gallusser 1999; De Vleeschouwer, *et al.*, 2000; French *et al.*, 1989; van Elsacker *et al.*, 1994).

We evaluated the potential to assess reproductive function in wild lion tamarins via fecal steroid monitoring. We tested whether the measures could document changes in reproductive status, e.g., pregnancy, in dominant breeding females. Further, we examined the reproductive status of several subordinate females—daughters living in natal groups and subordinate females residing in groups with unrelated dominant breeders—for a preliminary assessment of the impact of social environments on ovarian activity. We monitored reproductive endocrinology in dominant breeding females and daughters/subordinates in multiple social groups during 4 breeding seasons (1996 through 1999). The results demonstrate the utility of fecal steroid hormone monitoring in the species and in the context of wild tamarins, and confirm the complex seasonal, maturational, and social influences on reproduction in the golden lion tamarin.

METHODS

Focal Subjects and Study Site

We conducted all observations and sample collections at the Reserva Biológica de Poço das Antas, a 6300 ha reserve in southeastern Rio de Janeiro State, Brazil. The subjects were 10 dominant breeding females and 9 females that were natal daughters or behaviorally subordinate females residing in groups that contained a dominant, breeding female (Tables I and II). They resided in 9 different social groups. Exact birthdates are known for most females in the study because the groups are under intensive observation

Table I. Dominant reproductive females sampled in study

Female	# of Reproductively Active Females in group	Age	Group	No. of samples
3	2 (mother of #650)	16 y	GF	20
291	1	9–10 y	SA	84
436	2 (mother of #603)	6–7 y	2M	81
524	1	4–5 y	BA	41
539	1	4–5 y	AL	33
629	1	3 y	FA	16
651	1	4–5 y	2F	54
672	1	4 y	BO2	17
703	1	3 y	GF2	46
721	2 (dominant to #720; relationship unknown)	3 y	BO2	48
				Total Samples = 440

throughout the year (Dietz *et al.*, 1994; Dietz and Baker, 1993). For individuals that entered study groups from outside the marked population, we estimated age based on weight (for young individuals) and on tooth wear (for older animals). We determined intra-group dominance status among females from the incidence and direction of archwalks and chases, which indicate dominant status, and mounts, which indicate subordinate status, in the long-term behavioral records on the groups.

We collected samples from breeding females during a variety of phases of the annual reproductive cycle, including between and throughout pregnancy. Although we collected samples throughout the year, we focused on collecting feces from breeding and subordinate females during the breeding

Table II. Subordinate females sampled

Female	Group*	Status in Group	Age	# Sample
593	Intact Natal Group	Daughter	2 y	9
603	Nonintact Natal Group (Replacement Male)	Subordinate to mother	3–4 y	77
650	Intact Natal Group	Oldest daughter	2–3 y	41
685	Intact Natal Group	Daughter	1 y	26
688	Intact Natal Group	Daughter (twin of #689)	1 y	9
689	Intact Natal Group	Daughter (twin of #688)	1 y	49
704	Nonnatal Group	Subordinate to breeding female	1–2 y	5
720	Nonnatal Group	Subordinate to adult female, then dominant	2–3 y	58
723	Nonnatal Group	Emigrated with dominant sister	2 y	10

*Intact Natal Group = female resides in family with suspected mother and father present in group; Nonintact Natal Group = female resides in natal group with replacement breeder, male; Nonnatal Group = female has dispersed from natal group and joined group with suspected nonrelatives

season—mating, pregnancy, parturition, and lactation—from late May through early February. We sampled one subordinate female in 1996, but the remainder of the samples are from 1997, 1998, and 1999.

Sample Collection and Storage

Observers located radio-collared groups early in the morning, before emergence from the sleeping site. When tamarins left the sleeping sites, observers closely watched them for signs of defecation. Since concentrations of fecal progesterone metabolites (but not estrogen metabolites) vary in samples collected in the morning and afternoon in other callitrichid primates (Sousa and Ziegler, 1998), we attempted to restrict sample collection to mornings. We collected by 0900 h 50% of samples, and >80% by 1200 h. We included only samples that were unambiguous with regard to the animal of origin in the analyses. We collected a single sample from an individual on any given day. We placed the feces in a plastic whirl-pak sample bag, froze it within 3–6 h of collection, and stored it at -18°C until shipment to the U.S. on dry ice. Tests with fecal samples collected from captive callitrichids indicated that a delay in freezing fecal samples of up to 96 h post-collection did not alter progesterone metabolite concentrations >10%, relative to samples that were frozen immediately (Brewer and French, unpublished data).

Sample Extraction and Assay

Extraction

After thawing at room temperature we placed 0.125 g fractions of each sample in round-bottom 20-mL test tubes. We removed seeds, leafy material, and insect parts from the fecal sample before weighing. We added a 5-mL volume of solubilizer (40% methanol: 60% phosphate buffered saline (PBS)) and we placed the tubes on a shaker rack overnight (12–16 h). We pelleted particulate matter in the resulting slurry by centrifugation for 15 min at 2000 g, and separated the supernatant into a clean test tube. Recovery of tritiated steroid after extraction was $77 \pm 3.3\%$. We refroze and stored extracted samples before assay.

Enzyme Immunoassays (EIA)

We assessed fecal pregnanediol-3-glucuronide (PdG) levels via an EIA. The protocol was modified from French *et al.* (1996) as follows. We further

diluted extracted fecal samples with assay buffer (1:5) before measurement in order to place the sample concentration within the range of the standard curve. We constituted PdG standards (Sigma Chemical Co., St. Louis MO) in 1:5 extraction buffer:PBS, and they ranged from 10,000 to 78 pg/well in halving concentrations. We determined the precision of the fecal EIAs via a fecal quality control pool. The fecal quality control pool consisted of the supernatant of extracted fecal samples from several different females. Intra-assay coefficients of variation for high and low concentration quality controls were 10.2% and 6.8%. Interassay CVs were 11% and 14.4% for the high and low concentration pools, respectively. Serial dilutions of extracted feces from females at different times during pregnancy and PdG standards produced parallel displacement curves ($t(8) = 0.85, p > 0.40$). We determined the accuracy of the fecal PdG EIA by spiking the supernatant of an extracted female lion tamarin fecal sample with 2000 pg of PdG standard. The recovery of PdG was $108.6 \pm 5.9\%$ ($n = 6$).

We also measured fecal estrone conjugates (E_1C) by an EIA. The E_1C EIA was described by French *et al.* (1996), and we modified it as follows. We further diluted extracted fecal samples with assay buffer (1:20) before measurement in order to place the sample concentration within the range of the standard curve. We diluted E_1C standards (estrone-3-glucuronide, Sigma Chemical Co., St. Louis MO) in a 1:20 dilution of extraction buffer, and they ranged from 400 to 6.2 pg/well in halving concentrations. We determined the precision of the E_1C EIAs via the fecal quality control pool. Intra-assay CVs for high and low concentration pools were 4% and 3.6%. Interassay CVs based on the same pools were 22.1% and 19.6%, respectively. Serial dilutions of extracted feces from females at different times during pregnancy and E_1C standards produced parallel displacement curves ($t(8) = 0.91, p > 0.30$). We determined the accuracy of the assay by spiking the supernatant of an extracted female lion tamarin fecal sample with 25 pg of E_1C standard. The recovery of E_1C standard was $96.0 + 2.7\%$ ($n = 6$).

RESULTS

Normative Endocrine Function in Breeding Adult Females

Fecal steroid analyses on samples ($n = 440$ samples, 10 females) from breeding females in the Poço das Antas population revealed clear signs of ovulation, conception, and the onset of pregnancy. Figure 1 portrays reproductive profiles in adult females, showing the onset of ovarian function in June and July, with sustained and elevated hormone concentrations during pregnancy and declines in hormone concentrations following parturition.

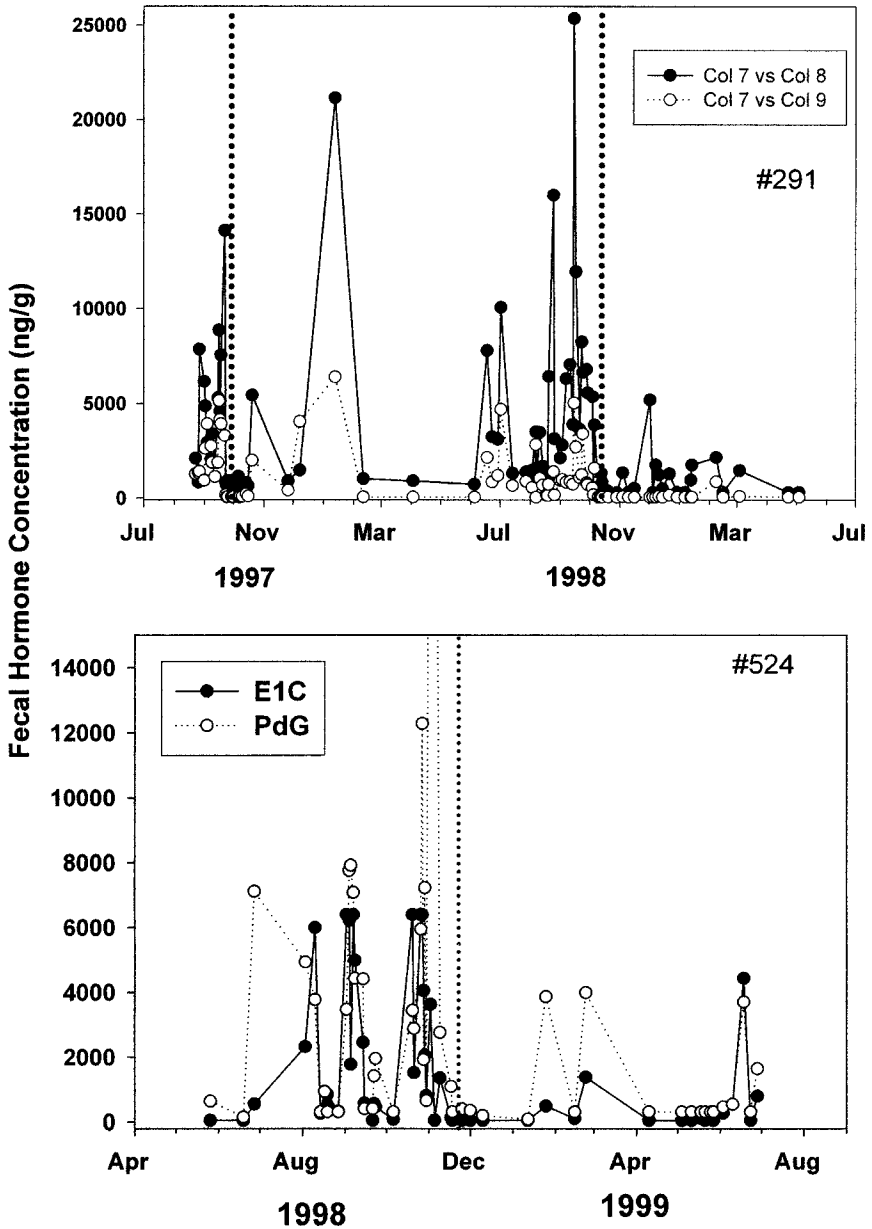


Fig. 1. Fecal steroid profiles for two dominant, breeding females in different social groups in Poço das Antas, across different reproductive phases. Vertical dotted lines represent dates of parturition.

Table III. Means (\pm standard errors) for fecal pregnanediol glucuronide (PdG) and estrogen conjugates (E₁C) for dominant reproductive females while non-pregnant and during each trimester of pregnancy (n = number of females sampled during the period)

Hormone (ng/g feces)	Non-pregnant ¹ (n = 10)	1st trimester ² (n = 7)	2nd trimester (n = 9)	3rd trimester (n = 10)
PdG	1052.32 \pm 172.82	2086.98 \pm 677.86	2905.18 \pm 1082.22	4467.64 \pm 884.93
E ₁ C	133.65 \pm 34.91	856.55 \pm 403.36	1133.19 \pm 273.82	1461.37 \pm 346.11

¹Pregnancy status was confirmed by back-dating from the day of parturition, assuming a 126 d gestation

²Trimesters were defined as three successive 42 d periods during pregnancy, back-dated from the day of parturition

The duration of elevated steroid concentration for both females is *ca.* 4 mo. Table III lists mean values for PdG and E₁C for non-pregnant females and females in each trimester of pregnancy. Although fecal sampling collection occurred less frequently outside the main breeding period, the low concentrations from February through June/July suggest a seasonal period of ovarian quiescence. Concentrations of both PdG and E₁C during pregnancy tended to be above >5000 ng/g feces, and reached levels as high as 10,000 – 15,000 ng/g (PdG) and 5,000 to 7,000 ng/g (E₁C). Pregnant females (dominant and subordinate) had significantly higher concentrations of estrogen and progesterone metabolites than when they were not pregnant, by at least one order of magnitude ($F(1, 11)'s > 23.70, p's < 0.001$).

Comparison of Endocrine Function in Dominant and Subordinate Females

Social status clearly influenced levels of ovarian hormone excretion in nonpregnant females. Figure 2 presents mean concentrations of PdG and E₁C measured in fecal samples collected from 9 subordinate females and 10 dominant breeding females in varying stages of reproduction. We contrasted mean concentrations of hormones in all samples from nonpregnant females (Fig. 2a and b) and from pregnant dominant and subordinate females (Fig. 2c and d) as a function of their status in the group: reproductively inactive subordinate, reproductively active subordinate, and dominant female. Levels of PdG excretion are significantly lower in subordinate females than in dominant females ($F(2, 15) = 4.76, p < 0.02$), regardless of whether subordinates ultimately became pregnant or not. While levels of E₁C tended to be lower in subordinates than in dominant females, the differences are not significant ($F(2, 15) = 1.55, n.s.$). Pregnancy status of females was clearly reflected in fecal concentrations of PdG and E₁C, (Fig. 2c and d). Pregnant subordinates had PdG concentrations that do not differ significantly from those of

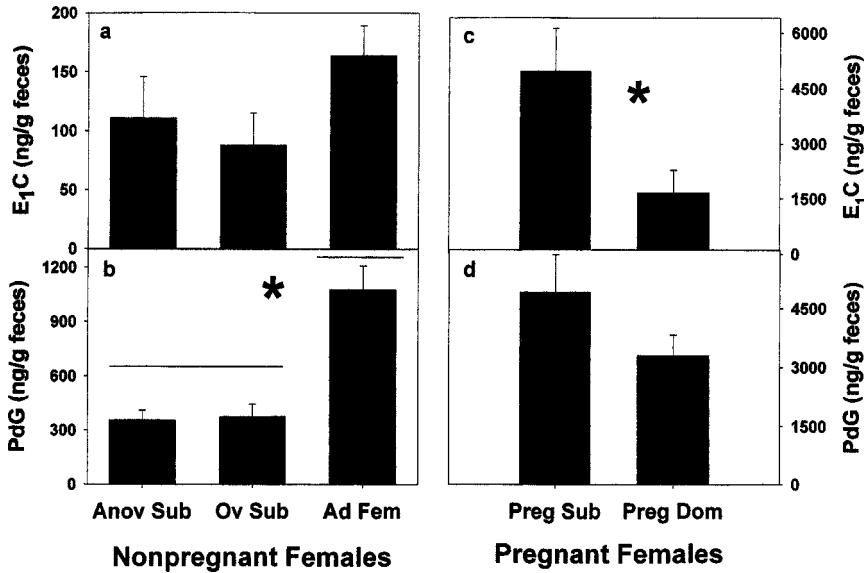


Fig. 2. Mean concentrations of fecal E₁C and PdG in females as a function of reproductive and social status in the group (anovulatory and ovulatory subordinates vs. dominant adult females (a and b) and for pregnant females that varied in social status (c and d). Note the different scales for the Y-axes on panels 3c and d. Anov Sub = noncycling subordinate female; Ov Sub = ovulatory subordinate; Ad Fem = dominant breeding female; Preg Sub = pregnant subordinate female; Preg Dom = pregnant dominant female.

dominant females ($F(1, 11) = 2.11, n.s.$). However, levels of E₁C are significantly higher in pregnant subordinates than levels in pregnant dominants ($F(1, 11) = 6.33, p < 0.03$).

Patterns of Fecal Steroid Excretion in Subordinate Females and Daughters

We collected 249 samples from 9 females identified as daughters or socially subordinate to a dominant breeding female. The females provided multiple samples (range = 5 – 77, median number of samples = 33.5), and we could therefore evaluate the resulting hormonal profiles qualitatively and quantitatively for signs of ovulation or pregnancy. In 5 females, there was clear evidence of anovulatory status during part or all of the sampling period. Figure 3 presents the profiles for 4 of them. Two of them (#685, #688, Fig. 3a, 3b) were 1-yr-old daughters living in intact natal groups, and most samples collected during this period had hormone concentrations at or just above the minimal level of sensitivity of the assays. Female #723 (Fig. 3c) had emigrated

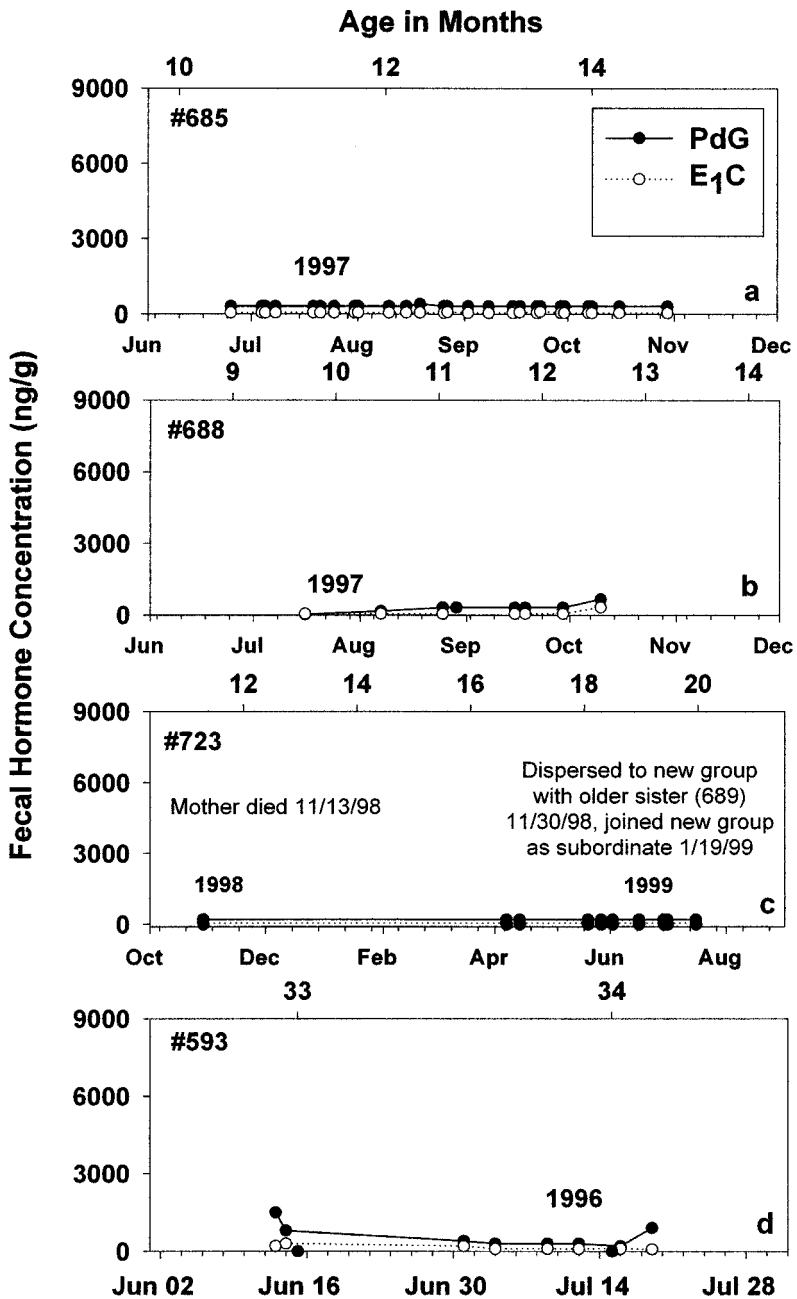


Fig. 3. Nonconceptive fecal steroid profiles for daughters and subordinate females in the presence of mothers or behaviorally-dominant females. For the duration of most of the sampling period for each female, there is little evidence for ovarian activity and/or pregnancy. See Table I and text for details on each female.

from her natal group as a 1-yr-old with her behaviorally-dominant sister (#689;) and joined a new group. Concentrations of fecal ovarian steroids for #723 never reached levels suggesting ovulatory function or pregnancy during the 1999 breeding season (Fig. 3c). Finally, there was no sign of ovarian activity for #593 (Fig. 3d), a 2-yr-old daughter living in an intact natal group.

Two females well past the age of puberty were apparently anovulatory while residing in groups as subordinate females, but ovulated and became pregnant when they became a dominant female in the group. Female #689 (Fig. 4a) was sampled as both a 1- and 2-yr-old while in her natal family group. In both years (1997 and 1998), she showed no evidence of ovulatory cycles or pregnancy. Female #689 dispersed with a younger sister at *ca.* 26 mo of age and joined a new group as a dominant female. Samples collected early in the breeding season revealed no sign of pregnancy in #689, though she might have produced a singleton on 10/4/1999. We also noted a period of steroid excretion levels suggesting anovulation for #720, a 4-yr-old subordinate female in a group she joined gradually from January through July 1998 (Fig. 4b). During the duration of her residence in the group as a subordinate (through February 1999), #720 showed low and acyclic concentrations of ovarian steroid excretion. However, her reproductive status changed when her social status changed. In February 1999, the dominant female left the group, and in May 1999, #720 displayed quantitative and qualitative steroid excretion profiles suggesting successful pregnancy. She delivered a set of twin infants on 9/28/99, which is 120 – 130 d after her hormone concentrations became elevated (Figure 4b).

Finally, 2 subordinate females displayed evidence of normal ovarian function, including conception and pregnancy while they resided in social groups in the presence of a dominant female. Their profiles are in Figure 5a and b. Female #650 (Fig. 5a) was a 2-yr-old daughter residing in her intact natal group with her mother, the oldest breeding female in the study population (16 yrs). Female #650 had steroid excretion profiles suggesting pregnancy, both in terms of the period of elevation (*ca.* 130 d) and in the concentrations of steroids (multiple samples > 3,000 ng/g feces). The dominant breeding female gave birth to infants on 9/25/97, after the date of apparent conception for #650. The dominant female died on 11/5/97, 5 weeks after the birth of her infants. The normal age of weaning in wild lion tamarins is *ca.* 12 weeks of age, and #650 was a primary carrier of the infants and also provided them with animal prey. She remained with the infants after her father and brother left the group in apparent search of a new mate. No infant born to #650 was seen by field staff, though the drop in steroid excretion in December suggests that her pregnancy was maintained for a normal gestation period.

Figure 5b portrays fecal steroid profiles for #603, a 3-yr-old in a group with her mother (#436) and multiple adult males that had entered the group

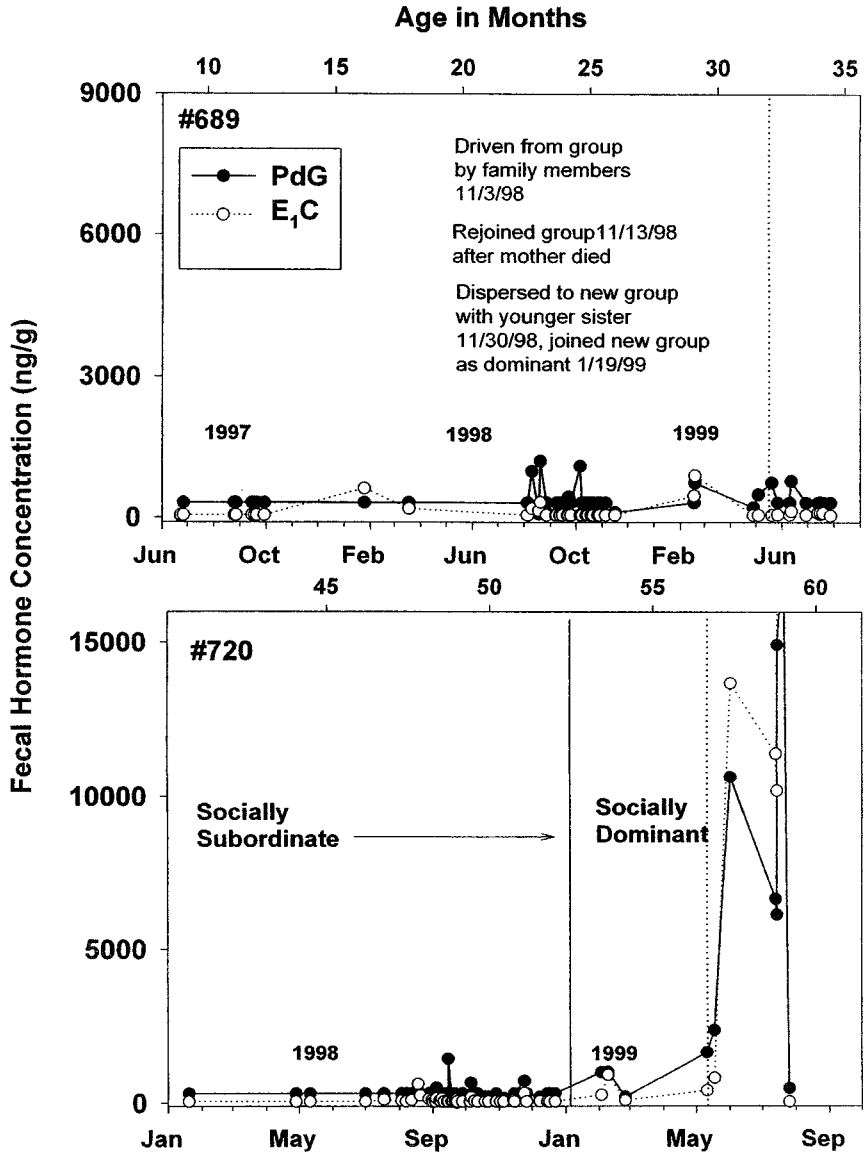


Fig. 4. Fecal steroid profiles in two females who displayed patterns suggesting infertility while residing as subordinates in social groups, but then exhibited an onset of reproductive function after changing groups (#689) and after changing status in a group (#720). Dotted vertical lines indicate estimated dates of conception for each female.

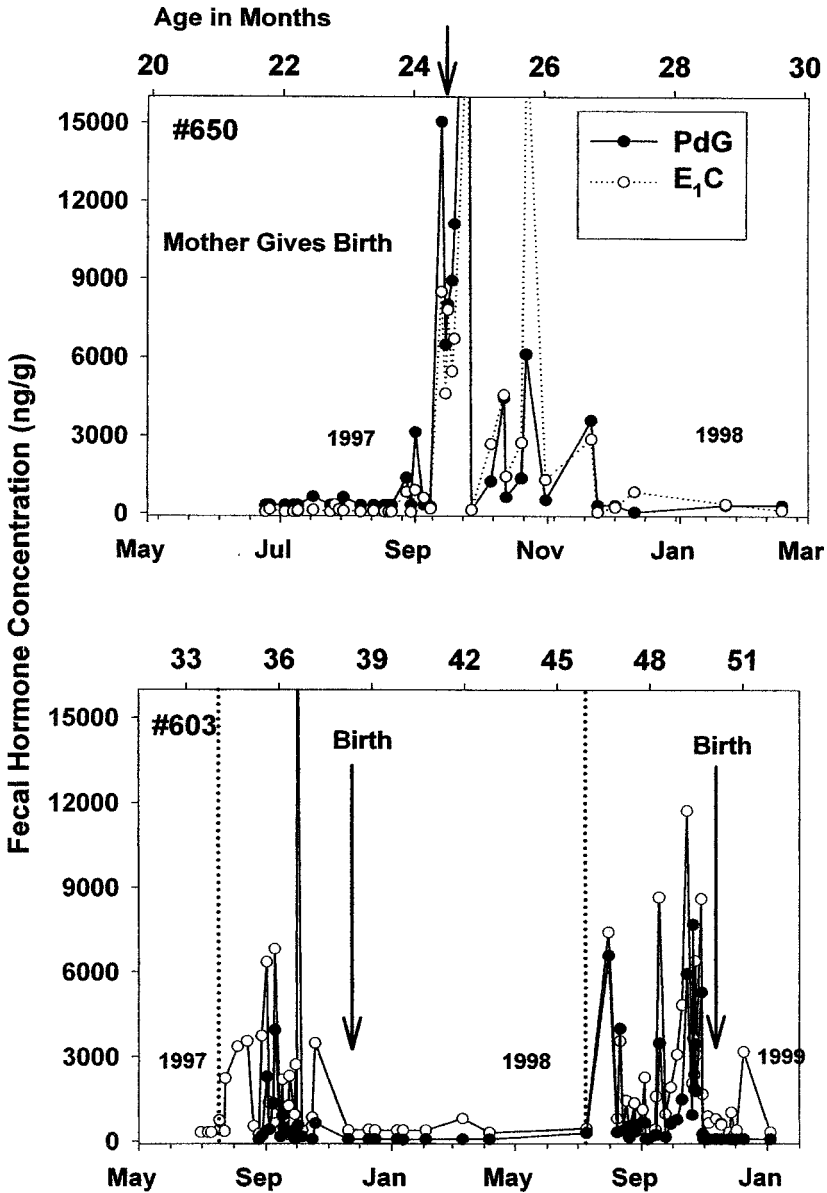


Fig. 5. Conceptive fecal steroid profiles for daughter residing in an intact family group (#650) and a subordinate female residing in her natal group with a replacement male (#603). Although the profile for #650 suggests pregnancy, no infants were observed for her. Dotted lines estimate the dates of conception, and solid vertical arrows indicate dates of parturition for female 603.

after 603's father died. Female 603 became pregnant twice (1998 and 1999), carried twins to term, and successfully reared both sets of infants. Concurrently her mother (#436, see Fig. 2a) also birthed and reared 2 sets of infants. Mother and daughter produced infants 30 days apart in 1997, and 10 days apart in 1998. In both cases, #436 delivered her litter first.

DISCUSSION

Our data clearly indicate that, as in other species (ungulates: Kapke *et al.*, 1999, carnivores: Creel *et al.*, 1992, 1997; neotropical primates: Strier and Ziegler, 1997; cercopithecine primates: Wasser *et al.*, 1988; great apes: Whitten *et al.*, 1998) useful information regarding female reproductive function can be acquired via the analysis of excreted steroid hormones in feces in lion tamarins. The profiles generated from breeding adult females clearly showed that we can differentiate pregnant from nonpregnant females on the basis of both qualitative and quantitative assessments. Daily collections under the controlled conditions of captivity can yield profiles that are useful to pinpoint the periovulatory phase of the ovarian cycle (French *et al.*, 2002). Under the conditions and demands of sample collection in the field, we were unable to collect samples with sufficient frequency to identify subtle details of the ovarian cycle during development (in subordinates) or during nonconceptive cycles (in breeders). However, the close agreement between periods of elevated steroids in dominant focal females (Fig. 1) and estimates of gestation length in captive lion tamarins (French and Stribley, 1985; Kleiman, 1978) clearly indicates the biological validity of the sampling schedule to monitor events between conception and parturition.

Although details of ovarian steroid hormone metabolism and excretion are known for urine in lion tamarins (French and Stribley, 1985), little is known about the metabolic fate of ovarian steroids excreted in their feces. Knowledge of steroid clearance in lion tamarins is critical, since there is considerable intergeneric variability in gut transit time and steroid hormone clearance rates among other callitrichid genera (*Saguinus* and *Callithrix*: Ziegler *et al.*, 1996). Further, recent work on squirrel monkeys suggests that estrogens and progestagens may be excreted in feces at different rates, with progestagens clearing more rapidly than estrogens (Moorman *et al.*, 2002). Knowledge of these parameters may increase our ability to resolve more subtle details of steroid hormone profiles in wild lion tamarins. Nevertheless our data are comparable to levels of fecal steroids from other wild callitrichid primates (cotton-top tamarins; Savage *et al.* 1997).

Dominant females differed from subordinates in several aspects of endocrine function. Among nonpregnant females, both anovulatory and

ovulatory subordinates had significantly lower concentrations of PdG than those of dominant adult females, and subordinates tended to have lower levels of E₁C than those of dominants. That anovulatory females have lower concentrations of ovarian steroids than dominants is not surprising; however, ovulatory subordinates also were clearly distinguishable from dominants on the basis of steroid hormone levels. In other callitrichid species in which subordinates ovulated, ovarian hormone profiles in the subordinates are also distinguishable from those of dominants, with lower peak levels of progesterone or progesterone metabolites on both and a shorter luteal phase duration (Saltzman *et al.*, 1994; Smith *et al.*, 1997).

Social status was not associated with differences in PdG concentrations, in pregnant females. However, pregnant subordinates had significantly higher levels of E₁C than those of pregnant dominant females. Subordinate females are likely attempting independent reproduction for the first time, while dominants varied in the number of previous litters, so it is possible that the differences do not reflect the influence of social status but instead portray parity or age effects. However, several dominant primiparous females in our sample displayed no unusually high E₁C value. Although we attempted to sample evenly throughout pregnancy, it is also possible that samples from pregnant subordinates were more likely to be collected at a time when hormone concentrations are high, e.g., second trimester, than at other times, but the lack of a difference in PdG concentrations argues against this interpretation. In any event, high prepartum E₁C is associated with traits associated with reduced reproductive performance. In pregnant wild lion tamarins, mothers with high prepartum E₁C have offspring with lower neonatal weights (Bales *et al.* 2002) and elevated estrogen concentrations are associated with lower maternal care and poor infant outcome in other callitrichids (Fite and French, 2000; *contra* Pryce *et al.*, 1988). Subordinate female lion tamarins that attempt reproduction have significantly lower reproductive success (estimated by the number of offspring surviving 6 mo) than dominants (Dietz and Baker, 1993), and the effects of elevated E₁C on reduced fetal growth and reduced maternal care may contribute to this effect.

Although our small sample of subordinate females limits our conclusions the endocrine profiles generated from the data suggest that ≥ 3 features influence reproductive function in daughters and subordinate females in groups of wild lion tamarins. Among them are age (younger females are likely to show patterns of infertility, older females are likely to show elevated steroid concentrations), composition of the group (older females in groups with both biological parents are less likely to show elevated steroid concentrations than females in groups with step-parents or no related adults), and relative dominance status among females (older females that are clear

social subordinates are less likely to display elevated steroid concentrations). It is difficult to disentangle the effects of age, nutritional status, and social status. However, as is increasingly apparent from both theoretical analyses of reproductive suppression in cooperatively breeding species (Creel and Waser, 1991; French 1997) and elegant experimental work on captive animals (Saltzman *et al.*, 1997a, 1997b; Widowski *et al.*, 1990), reproductive suppression in female callitrichids is a physiological response that is sensitive to a complex suite of variables.

As in captive studies on other callitrichid primates, age was a significant predictor of whether a subordinate female tamarin showed ovarian activity. Only one of the 4 females that were sampled when they were <16 mo old showed signs of elevated hormone excretion (#704), and she had only a single elevated sample with no sign before or after of continued activity. In addition, she was one of the females whose birthdate was estimated rather than known; therefore, she might have been older than estimated. Data on reproductive ontogeny in captive female lion tamarins residing in intact family groups also suggest that females <14 mo old have low and acyclic patterns of urinary estrogen excretion (French, 1997; French *et al.*, 1989; French and Stribley, 1985). In the Poço das Antas population, no female in her first year has ever conceived offspring. Together, these observations suggest that the pattern of low and acyclic concentrations of steroid excretion in 1-yr-old females is not socially-mediated, but instead reflects a prepubertal state in the female. However, low and acyclic steroid excretion profiles in older females (>20 mo: #723, #593, #689, #720) probably reflect social influences on ovarian function.

Only one female (#650) conceived while residing in a natal group in which no unrelated male was present. Her 16-yr-old mother was the oldest focal female at Poço das Antas. Although the latter was pregnant and gave birth during the sampling period, she was in poor health and died 5 weeks postpartum. Further, before her death she did not engage in the social role typical of a dominant female, such as leading group movement and participating in agonistic responses during intergroup encounters. Indeed, #650 was among the most aggressive group members during the territorial encounters. Recently, examples of subordinate reproduction in several species suggest that subordinate females are sensitive to the reproductive or health status of the dominant female, and the onset of independent reproduction is based on these assessments. For example, in common marmosets, subordinate female ovulation was common (8 of 10) in family groups where the mother was ovulating but did not produce viable offspring, but subordinate female ovulation was much less common (4 of 16) when the mother was regularly conceiving and carrying infants to term (Saltzman *et al.* 1997a). Perhaps subordinate female lion tamarins are sensitive to the dominant's reproductive potential, and are more likely to both ovulate and to conceive

when the dominant is old or anovulatory. Further data on subordinate females in groups with aging or sick dominant females will help to address this possibility.

Our endocrine data, together with long-term field records from Poço das Antas (Baker *et al.* 2002) suggest that subordinate females may choose whether to reproduce. Breeding records indicate that among 2-yr-old females, not a single female (of 17) that was co-resident in a group only with related males (fathers and/or brothers) produced offspring, though our endocrine data presented suggest that one female may have been pregnant. In contrast, 3 of 8 2-yr-old subordinates that lived in groups with ≥ 1 unrelated males produced offspring. If subordinates continue to reside in intact natal groups, the likelihood of pregnancy increases: 55% of 3-yr-old females, and all 4-yr-old females, became pregnant while living in groups that contained only related males. There are a variety of mechanisms that could produce this age-graded choice among subordinate females. First, it may be that stimulation from unrelated males is more critical for the onset of reproductive function in younger females than in older females (French *et al.*, 1984; Heistermann *et al.*, 1989; Widowski *et al.*, 1990), and thus older subordinates commence ovulation in the absence of unfamiliar males. Secondly, older females may be more successful in seeking and achieving extragroup copulations from neighboring males (Digby, 1999) than younger females are, and subsequently have a higher rate of pregnancy. Finally, as females grow older and residual reproductive value continues to decrease, subordinate females may become more tolerant of incestuous matings. This option may reflect a better choice for an older subordinate, which may run the risk of missing another breeding opportunity. The benefits of this option are considerably enhanced when considered in light of the high costs of female dispersal (Baker and Dietz, 1996). Knowledge about the paternity of offspring born to subordinate females would help differentiate among possible mechanisms.

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