

Endocrine Monitoring of Dominant and Subordinate Females in Free-ranging
Golden Lion Tamarins (*Leontopithecus rosalia*)

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ABSTRACT

In captive callitrichid primates, reproductive function in females tends to vary with social status. However, little is known about the interplay among these factors in females living in wild groups. Here we report observations on normative ovarian function in dominant and subordinate female golden lion tamarins (*Leontopithecus rosalia*) living in wild groups. Ovarian status was monitored by measuring, via enzyme immunoassay, concentrations of excreted pregnanediol glucuronide (PdG) and estrone conjugates (E₁C) in fecal samples collected noninvasively from animals living 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 approximately 4 months, 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. Endocrine profiles consistent with ovulation and subsequent pregnancy were noted for behaviorally subordinate females living in groups with unrelated males or in which a reversal in female dominance status was noted. Our results suggest that in addition to changes in female reproductive endocrinology associated with puberty, the regulation of reproduction in females in wild callitrichid groups can be sensitive to status and relatedness to breeding males.

Keywords: 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 (marmosets and tamarins), a variety of mechanisms can produce this dramatic reproductive skew among females. These mechanisms include delay of puberty (French *et al.*, 1984; Savage *et al.*, 1988; Küderling *et al.*, 1995; Carlson *et al.*, 1997; Saltzman *et al.* 1997a), suppression of ovulation and ovarian production of steroid hormones in older subordinates (French *et al.* 1984; Abbott and George, 1991; Smith *et al.*, 1997), and behaviorally-mediated mechanisms such as agonistic interactions or inhibition of sexual behavior (“incest taboo”; French and Inglett, 1989; 1991; Saltzman *et al.*, 1997b).

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, 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. On the other hand, without the constraints 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 (e.g., Digby and Ferrari, 1994; Digby 1999). Under these conditions, then, we might expect less pronounced inhibition among subordinate females.

The development of noninvasive hormone monitoring methodologies (see review in Whitten *et al.* 1998) has allowed workers in reproductive biology 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; Ziegler *et al.*, 1987; Savage *et al.*, 1988). 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 using noninvasively collected urine samples. Nonconceptive ovarian cycle length is 19.6 days, and endocrine profiles suggest a gestation length of 125 days (French and Stribley 1985; French *et al.* 2002). 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 months; French *et al.* 1989; see also van Elsacker *et al.* 1994; Chaoui and Hasler-Gallusser 1999, and De Vleeschouwer, *et al.* 2000 for similar examples from other lion tamarins). In the present paper, we evaluated the potential for assessing reproductive function in wild lion tamarins through fecal steroid monitoring. We tested whether these measures could document changes in reproductive status (e.g., pregnancy) in dominant breeding females. Further, we examined the reproductive status of a number of 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 four breeding seasons (1996 through 1999). The results demonstrate the utility of fecal steroid hormone monitoring in this species and in this context of free-ranging tamarins, and confirm the complex seasonal, maturational, and social influences on reproduction in the golden lion tamarin.

METHODS

Focal Subjects and Study Site

All observations and sample collections were carried out at the Reserva Biológica de Poço das Antas, a 6300 hectare reserve in southeastern Rio de Janeiro State, Brazil. The subjects for this study 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 1 and 2). These females resided in 9 different social groups. Exact birthdates are known for most females in the study since the groups are under intensive observation throughout the year (e.g., Dietz and Baker, 1993; Dietz *et al.*, 1994). For animals that entered study groups from outside the marked population, age was estimated based on weight (for young individuals) and on tooth wear (for older animals). Within-group dominance status among females was determined from the incidence and direction of archwalks and chases (indicative of dominance status) and mounts (indicative of subordinate status) in the long-term behavioral records on the groups.

Samples from breeding females were collected during a variety of phases of the annual reproductive cycle, including between and throughout pregnancy. While samples were collected throughout the year, we focused on collecting feces from breeding and subordinate females during the midst of the breeding season, from late May through early February. One subordinate female was sampled in 1996, but the remainder of the females provided samples in 1997, 1998, and 1999.

Sample Collection and Storage

Observers located radio-collared groups early in the morning, prior to emergence from the sleeping site. When tamarins left their sleeping sites, observers closely watched all animals in the

group for signs of defecation. Since concentrations of fecal progesterone metabolites (but not estrogen metabolites) vary in samples collected in the mornings and afternoons in other callitrichids primates (Sousa and Ziegler, 1998), we attempted to restrict sample collection to morning defecations. Fifty percent of all samples were collected by 0900 h, and over 80% were collected by 1200 h. Only samples that were unambiguous with regard to the animal of origin were included in these analyses. A single sample was collected from an individual on any given day. Upon collection, the fecal samples were placed in a plastic “whirl-pak” sample bag, frozen within 3 –6 hours of collection, and stored at -18° C until shipment to the U.S. on dry ice. Tests with fecal samples collected from captive callitrichids indicate that a delay in freezing fecal samples of up to 96 hours post-collection has no effect on steroid concentration as measured by our assays (Brewer and French, in prep).

Sample Extraction and Assay

Extraction. Fecal samples were allowed to thaw at room temperature, and 0.125 g fractions of each sample were placed in round-bottom 20 ml test tubes. Seeds, leafy material, and insect parts were removed from the fecal sample prior to weighing. A 5.0 ml volume of solubilizer (40% methanol: 60% phosphate buffered saline (PBS)) was added and the tubes were placed on a shaker rack overnight (12 – 16 h). Particulate matter in the resulting slurry was pelleted by centrifugation for 15 min at 2000 *g*, and the supernatant was separated into a clean test tube. Extracted samples were refrozen and stored prior to assay.

Enzyme Immunoassays (EIA).

Fecal pregnanediol-3-glucuronide (PdG) levels were assessed using an EIA. The protocol was modified from French *et al.* (1996) as follows. Microtiter plates (Nunc Maxisorp F96) were coated with rabbit anti-PdG-BSA (P26) diluted 1:20,000 in sodium carbonate coating buffer. Extracted fecal samples were further diluted with assay buffer (1:5) prior to measurement in order to place the sample concentration within the range of the standard curve. PdG standards (Sigma Chemical Co., St. Louis MO) were diluted 1:5 in extraction buffer, and ranged from 10,000 to 78 pg/well in halving

concentrations. PdG-horseradish peroxidase (HRP) conjugate (batch 5/95) was diluted 1:20,000 in PBS and added to the wells. After a 2 h incubation, plate wells were washed and hydrogen peroxide and ABTS (2,2'-Azino-bis(3-thylbenzthylene-6-sulfonic acid)) was added as a chromogen. Absorbance was measured one hour later at 410 nm (reference 570 nm) in a Dynatech MR5000 microplate reader, and sample concentrations were calculated using a 4-parameter sigmoidal curve fitting function. The precision of the fecal EIAs was determined using a fecal quality control pool. The fecal quality control pool consisted of the supernatant of extracted fecal samples from several different females. Intra- and interassay coefficients of variation for a high and low concentration urine quality controls were 10.2% and 11.0% (high) and 6.8% and 14.4% (low), respectively. Serial dilutions of extracted feces from females at different times during pregnancy and PdG standards produced parallel displacement curves. The accuracy of the fecal PdG EIA was determined 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).

Fecal estrone conjugates (E₁C) were also measured by an EIA. The E₁C EIA described in French *et al.* (1996) was modified as follows. Microtiter plates (Nunc Maxisorp F96) were coated with rabbit anti-E₁C-BSA (R522) diluted 1:15,000 in sodium carbonate coating buffer. Extracted fecal samples were further diluted with assay buffer (1:20) prior to measurement in order to place the sample concentration within the range of the standard curve. E₁C standards (estrone-3-glucuronide, Sigma Chemical Co., St. Louis MO) were diluted in a 1:20 dilution of extraction buffer, and ranged from 400 to 6.2 pg/well in halving concentrations. E₁C-horseradish peroxidase (HRP) conjugate (batch 5/95) was diluted 1:20,000 in PBS and added to the wells. After a 2 h incubation, plate wells were washed and hydrogen peroxide and ABTS added as a chromogen. Absorbance was measured one hour later as per the PdG assay.

The precision of the fecal EIAs was determined using the fecal quality control pool. Intra- and interassay coefficients of variation for a high and low concentration controls were 4.0% and 22.1%

(high) and 3.6% and 19.6% (low), respectively. Serial dilutions of extracted feces from females at different times during pregnancy and E₁C standards produced parallel displacement curves. The accuracy of the assay was determined by spiking the supernatant of an extracted female lion tamarin fecal sample with 25 pg of E₁G standard. The recovery of E₁G standard was $96.0 \pm 2.7\%$ (n=6).

RESULTS

Normative Endocrine Function in Breeding Adult Females

Fecal steroid analyses on samples (n = 440 samples, 10 females) collected 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 two adult females, showing the onset of ovarian function in the months of June and July, with sustained and elevated hormone concentrations during pregnancy, and declines in hormone concentrations following parturition. The duration of elevated steroid concentration for both females is approximately 4 months. Table 2 presents 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 they did when they were not pregnant, by at least one order of magnitude (F(1,11)'s > 23.70, p's < 0.001).

Quantitative 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 nine 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 were 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 were not significant ($F(2,15) = 1.55$, n.s.). Pregnancy status of females was clearly reflected in fecal concentrations of PdG and E₁C, and concentrations are shown in Fig. 2c and d. Pregnant subordinates had PdG concentrations that did not differ significantly from those of dominant females ($F(1,11) = 2.11$, n.s.). However, levels of E₁C were significantly higher in pregnant subordinates than levels in pregnant dominants ($F(1,11) = 6.33$, $p < 0.03$).

Qualitative Patterns of Fecal Steroid Excretion in Subordinate Females and Daughters

A total of 249 samples was collected from nine females identified as daughters or socially subordinate to a dominant breeding female. These 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 five females, there was clear evidence of anovulatory status during part or all of the sampling period. Figure 3 presents the profiles for four of these females. Two of the four females (685, 688, Fig. 3a, 3b) were one-year-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) was a female who emigrated from her natal group as a one-year-old with her behaviorally-dominant sister (689; see below) and joined a new group. Concentrations of fecal ovarian steroids for 723 never reached levels suggestive of ovulatory function or pregnancy during the 1999 breeding season (Fig. 3c). Finally, no signs of ovarian activity were noted for female 593 (Fig. 3d), a two-year-old daughter living in an intact natal group. Female 704, a one-year-old subordinate in a nonnatal family group, produced one sample with elevated hormone concentrations in late November 1997 (PdG: 4,201 ng/g,

E₁C: 541 ng/g), but her samples before and after this date did not have detectable steroid concentrations (data not shown).

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 the dominant female in the group. Female 689 (Fig. 4a) was sampled as both a one- and two-year-old while in her natal family group. In both years (1997 and 1998) there was no evidence of ovulatory cycles or pregnancy. Female 689 dispersed with a younger sister at approximately 26 months of age and joined a new group as a dominant female. Samples collected early in the breeding season revealed no signs of pregnancy in 689, although she is suspected of producing a singleton infant on 10/4/1999. A period of steroid excretion levels suggesting anovulation was also noted for female 720, a four-year-old subordinate female in a group she joined gradually from January through July 1998 (Fig. 4b). During the duration of her residence in this group as a subordinate (through February 1999), 720 showed low and acyclic concentrations of ovarian steroid excretion. However, 720's 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 suggestive of successful pregnancy. 720 delivered a set of twin infants on 9/28/99, which is 120 – 130 days after hormone concentrations became elevated in 720 (see Figure 4b).

Finally, two 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. These profiles are shown in Figure 5a and b. Female 650 (Fig. 5a) was a two-year-old daughter residing in her intact natal group together with her mother, 3, the oldest breeding female in the study population (16 years old). Female 650 showed steroid excretion profiles suggestive of pregnancy, both in terms of the period of elevation (~ 130 days) 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, five weeks after the birth

of her infants. The normal age of weaning in wild lion tamarins is approximately 12 weeks of age, and we noted that 650 was a primary carrier of the infants and also provided them with animal prey items. Female 650 remained with the infants after her father and brother left the group in apparent search of a new female mate. No infants produced by 650 were ever seen by field staff, although the drop in steroid excretion in December suggests the pregnancy was maintained for a normal gestation period.

Figure 5b portrays fecal steroid profiles for Female 603, a 3-year-old female living in a group with her mother (436) and multiple adult males who had entered the group after 603's father died. 603 became pregnant on two occasions (once in 1998 and again in 1999), carried twins to term, and successfully reared both sets of infants. Her mother (436, see Fig. 2a) also successfully produced and reared two sets of infants during this period. 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 (e.g., ungulates: Kapke *et al.*, 1999; carnivores: Creel *et al.*, 1992; Creel *et al.*, 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 through the analysis of excreted steroid hormones in feces in lion tamarins. The profiles generated from breeding adult females clearly showed that we could 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 in pinpointing 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 females in this study (Fig. 1) and estimates of gestation length

in captive lion tamarins (Kleiman 1978; French and Stribley 1985) clearly indicates the biological validity of this 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 the feces in this species. 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 genera of callitrichids (e.g., *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. Regardless, however, our data are comparable to levels of fecal steroids noted in other free-ranging callitrichid primates (e.g., 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 dominant adult females, and subordinates tended to have lower levels of E1C than 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 have been reported to ovulate, ovarian hormone profiles in the subordinates are also distinguishable from those of dominants, with lower peak levels of progesterone and/or progesterone metabolites 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 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 these differences do not reflect the influence of social status but rather portray parity or age effects. However, several dominant primiparous females were included in the sample and these females did not display unusually high E_1C values. Although attempts were made 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 E_1C is associated with traits associated with reduced reproductive performance. In pregnant wild lion tamarins, mothers with high prepartum E_1C 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; but see Pryce *et al.*, 1988). Subordinate female lion tamarins that attempt reproduction have significantly lower reproductive success (estimated by the number of offspring surviving to six months of age) than dominants (Dietz and Baker 1993), and the effects of elevated E_1C on reduced fetal growth and reduced maternal care may contribute to this effect.

Although our small sample of subordinate females limits the conclusions we are able to draw, the endocrine profiles generated from these data suggest that at least three features influence reproductive function in daughters and subordinate females in groups of free-ranging lion tamarins. Among these features are age (younger females are likely to show patterns of infertility, older females are likely to show elevated steroid concentrations), composition of the group (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 (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 (e.g., Creel and Waser, 1991; French 1997) and elegant experimental work on captive animals (e.g.,

Widowski *et al.*, 1990; Saltzman *et al.*, 1997a; 1997b), 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 or not a subordinate female tamarin showed ovarian activity. Only one of the four females that was sampled when they were less than 16 months of age showed signs of elevated hormone excretion (704), and she had only a single elevated sample with no signs before or after of continued activity. In addition, this was one of the females whose birthdate was estimated rather than known; she therefore might have been older than estimated. Data on reproductive ontogeny in captive female lion tamarins residing in intact family groups also suggests that females younger than 14 months of age have low and acyclic patterns of urinary estrogen excretion (French and Stribley, 1985; French *et al.*, 1989; French, 1997). 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 one-year-old females is not socially-mediated, but rather reflects a prepubertal state in the female. However, low and acyclic steroid excretion profiles in older females (>20 months of age; #723, #593, #689, #720) probably reflect social influences on ovarian function.

Only one female in our study (650) conceived while residing in a natal group in which no unrelated males were present. Her mother was the oldest female under study at Poço das Antas, at 16 years of age. Although the mother was pregnant and gave birth during the sampling period, she was in poor health and died 5 weeks post-partum. Female 3 had great difficulty capturing or processing high quality animal prey, and her fecal samples were characterized by high-seed content. Further, 3 no longer engaged in the social role typical of a dominant female, such as leading group movement and coordinating agonistic responses during intergroup encounters. Indeed, the daughter 650 was among the most aggressive group members during these territorial encounters. Recently, examples of subordinate reproduction in several species suggest that these females are sensitive to the reproductive and/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 (eight of 10) in family groups where the mother was ovulating but did not produce viable offspring, but subordinate female ovulation was much less common (four of 16) when the mother was regularly conceiving and carrying infants to term (Saltzman *et al.* 1997a). It may be that subordinate female lion tamarins are sensitive to the dominant's reproductive potential, and are more likely to both ovulate and conceive when the dominant is old or anovulatory. Further data on subordinate females in groups with aging or sick dominant females will help address this possibility.

Our endocrine data, together with long-term field records from the Poço das Antas field site (Baker and Dietz, in preparation), suggest that subordinate females may “choose” whether to reproduce. Breeding records indicate that among two-year-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 (although the endocrine data presented in this paper suggests that one female may have been pregnant). In contrast, three of eight two-year-old subordinates that lived in groups with one or more unrelated males produced offspring. If subordinates continue to reside in intact natal groups, the likelihood of pregnancy increases (55% of three-year-old females, and all four-year-old females, became pregnant while living in groups that contained only related males). Regardless of age, subordinate females were much more likely to become pregnant if their social group contained unrelated 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 extra-group copulations from neighboring males (e.g., Digby, 1999) than are younger females, 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

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incestuous matings. This option may reflect a better choice for an older subordinate, who 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 the mechanisms described above.

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Table 1. 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

Table 2. Subordinate females sampled in study.

Female	Group*	Status in Group	Age	# Samples
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
 Nonnatal Group = female has dispersed from natal group and joined group with suspected nonrelatives

Table 3. 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 (n=10)	1 st trimester (n = 7)	2 nd trimester (n = 9)	3 rd 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

Figure Legends

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

Figure 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.

Figure 3 a – d. 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 period, there is little evidence for ovarian activity and/or pregnancy. See Table I and text for details on each female.

Figure 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.

Figure 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.

Fecal Hormone Concentration (ng/g)









