The steroid hormone cortisol has been associated with different levels of “stress” as well as different reproductive conditions in many primates. In callitrichids, cortisol has more often been reflective of female reproductive status than of chronic stress. In this study, we addressed the hypothesis that wild golden lion tamarin (Leontopithecus rosalia) females, whose social structure is characterized by low aggression and high social support, would not show rank (“stress”)–related differences in glucocorticoids but would show reproductive changes. We collected 710 fecal samples from 22 adult females in Poço das Antas Reserve, Brazil, and nearby reintroduction areas, and assayed them for cortisol. Differences in cortisol levels were found between different reproductive conditions. Females in the first trimester of pregnancy had lower cortisol levels than nonpregnant females, although we did not differentiate between basal and ovulating levels in nonpregnant females. Cortisol rose in the third trimester of pregnancy. Primiparous females had a higher rise in the third trimester than multiparous females. No differences in cortisol levels were found among dominant females, ovulatory subordinate females, or anovulatory subordinate females. These results are similar to those obtained in other studies of callitrichid females. The lack of differences in cortisol excretion between dominants and subordinates is likely due to the low levels of overt aggression and the high level of social support available to subordinate females. Am J Primatol 67:25–35, 2005.

Key words: cortisol; tamarin; callitrichid

Contract grant sponsor: NSF; Contract grant numbers: SBR-9828687; BCS-0216096; Contract grant sponsor: University of Maryland; Contract grant sponsor: Sigma Xi; Contract grant sponsor: Friends of the National Zoo; Contract grant sponsor: NSF Biology of Small Populations Research Training Grant.

Correspondence to: Karen L. Bales, Dept. of Psychology, University of California–Davis, Davis, CA 95616. E-mail: klbales@ucdavis.edu

Received 15 September 2004; revised 3 January 2005; revision accepted 7 January 2005

DOI 10.1002/ajp.20167
Published online in Wiley InterScience (www.interscience.wiley.com).

© 2005 Wiley-Liss, Inc.
INTRODUCTION

In many primate species, levels of the steroid hormone cortisol are affected by a number of factors, including social status [Cavigelli et al., 2003; Saltzman et al., 1994; Sapolsky, 1995; Shively et al., 1997], social stability [Sapolsky, 1983; Smith et al., 1997], stressful experiences [Albuquerque et al., 2001; Johnson et al., 1996; Smith & French, 1997a, b], and reproductive stage [Weingrill et al., 2004; Ziegler & Sousa, 2002; Ziegler et al., 1995]. Cortisol levels can also vary both across and within taxonomic groups [Abbott et al., 2003].

Abbott et al. [2003] used a meta-analysis of primate studies to examine the relationship between dominance status and activity in the hypothalamic-pituitary-adrenal (HPA) axis. The species examined included the common marmoset (Callithrix jacchus), cotton-top tamarin (Saguinus oedipus), squirrel monkey (Saimiri sciureus), rhesus macaque (Macaca mulatta), talapoin (Miotopithecus talapoin), cynomolgous macaque (Macaca fascicularis), and olive baboon (Papio anubis). The authors examined a number of factors that might predict the ratio of basal cortisol concentrations expressed by subordinates relative to concentrations expressed by dominants. Factors identified with higher levels of cortisol for subordinates included higher rates of stressors and lower rates of social support. In contrast, in those species and situations in which subordinates experienced lower exposure to potential stressors and/or had higher levels of social support, subordinates tended to have cortisol levels similar to those of dominant individuals. With knowledge of these social parameters, it is possible to make predictions regarding relative levels of cortisol for dominants and subordinates in a given primate species.

These predictions are confirmed by available data on females from several callitrichid species (mostly captive populations). In general, increases in cortisol appear to be connected to reproductive events and endocrine status rather than to chronic stress. In a study of wild female common marmosets [Albuquerque et al., 2001], cortisol levels were elevated during late pregnancy. A study of captive common marmosets [Ziegler & Sousa, 2002], which also showed a rise in cortisol in late pregnancy, found no differences in cortisol between mothers (dominants) and daughters (subordinates) when they were directly compared during the same period. In captive cotton-top tamarins [Ziegler et al., 1995], reproductively suppressed subordinate females in their natal groups had lower levels of cortisol than their mothers, and ovarian activity was associated with higher cortisol levels. Captive Wied’s tufted ear marmosets (Callithrix kuhliii) also displayed elevated cortisol during the third trimester of pregnancy, with no mean differences in cortisol levels between dominant and subordinate females [Smith & French, 1997b]. However, cortisol in both common marmosets and Wied’s tufted-ear marmosets has also been shown to be sensitive to psychogenic stressors caused by experimental manipulations such as changes in the social group [Johnson et al., 1996; Smith et al., 1997; Ziegler & Sousa, 2002], isolation [Johnson et al., 1996; Smith & French, 1997a, b], and manual restraint [Smith & French, 1997b].

The relationship of cortisol to reproductive and social status has also been successfully addressed in other wild, non-callitrichid primate subjects. In female ring-tailed lemurs (Lemur catta), female subordinates are reproductive, and therefore social status and reproductive state are not confounded. In one study, fecal cortisol levels were found to be high during late pregnancy, as well as in dominant individuals [Cavigelli, 1999]. In dominant individuals, higher cortisol values were associated with the initiation of aggression, while higher cortisol
values in subordinates were associated with receipt of aggression [Cavigelli et al., 2003]. In contrast, in free-ranging chacma baboons (Papio hamadryas ursinus), Weingrill et al. [2004] found no relationship between cortisol and social rank, but reported that estrous females had low cortisol levels. In another study, wild female muriquis had lower cortisol levels during mating-only periods vs. premating and conception periods [Strier et al., 2003].

In this study we examined the cortisol profiles of free-ranging female golden lion tamarins in Rio de Janeiro State, Brazil. Golden lion tamarins (Leontopithecus rosalia) have a cooperative-breeding social structure in which (usually) one female breeds with one or more males [Baker et al., 1993]. Polygyny occurs in about 10% of group-years and usually involves a mother–daughter pair in which the breeding male has been replaced [Dietz & Baker, 1993]. Both males and older offspring help rear infants. Aggression is rare (rather than continuous) and is usually associated with dispersal events. Relationships between subordinate and dominant females are often characterized by high levels of mutual affiliative behavior [Baker et al., 2002]. Given the predictors of status differences in cortisol identified by Abbott et al. [2003], we predicted that subordinate female lion tamarins would express cortisol levels similar to or less than those expressed by dominant females. It is possible that cortisol concentrations would be lower in subordinates if they were anovulatory while dominants were reproductive. Although reproduction in captive female golden lion tamarins is not physiologically suppressed [French, 1987; French et al., 2002], in wild groups there is evidence that some adult subordinate females experience anovulation [French et al., 2003].

MATERIALS AND METHODS

Subjects

The subjects were 22 female golden lion tamarins (13 dominants, five subordinates, and four that were both dominant and subordinate). The females ranged freely in Poço das Antas Biological Reserve (PDA) and outlying reintroduction areas (Table I). Drs. James Dietz and Andrew Baker, and colleagues have monitored approximately 20 groups in the PDA population since 1983. All individuals in the study groups are tattooed and dye-marked, and at least one animal in each group is radiocollared to facilitate location.

The females were classified as dominant if they were the only breeding female in the group or were behaviorally dominant to another female (based on access to the breeding male, arch-walks, mounts, and chases). They were classified as ovulatory or anovulatory based on levels of fecal estrogen conjugates and pregnanediol glucoronide [French et al., 2003]. Subordinate females, particularly those that were anovulatory, tended to be younger than dominant females (dominant females: age (mean ± standard error)=1909.65 ± 289 days; ovulatory subordinates: age=1443 ± 386 days; anovulatory subordinates: age=604 ± 205 days). However, age was not significantly associated with cortisol levels in an analysis of covariance (F1.24=2.33, P=0.14). In addition, the overall pattern of results did not change when samples taken before the females were 18 months old were excluded.

Four dominant females were descendants of captive-born animals that had been reintroduced from zoos [Beck & Martins, 1998]. These females were all born in the wild and were provisioned three times a week. Descendants of reintroduced monkeys are capable of foraging independently and behave in many respects like native wild tamarins. These females did not differ from the PDA females in
baseline cortisol levels (Mann-Whitney test, U=43.0, P=0.303), and the two groups were therefore combined for all analyses.

**Collection, Extraction, and Assay of Fecal Samples**

We collected fecal samples approximately twice a week for each female during four periods: June–July 1996 (preliminary data), June–November 1997, July–December 1998, and February–March 1999. Samples were also collected opportunistically during other periods. Since cortisol concentrations in feces vary depending on the time of day [Sousa & Ziegler, 1998], we attempted to restrict the sample collection to mornings. We collected 50% of the samples by 9:00 a.m., and 80% by 12 p.m. We used only samples that were unambiguous as to the identity of the animal of origin, and included only one sample per animal per day in the analysis. The number of samples differed among females due to circumstances such as death, formation of new study groups, and an unexpected birth by one subordinate female. The samples were stored at −18°C until they were analyzed. No more than 10 hr passed between the collection and freezing of the samples. The samples were kept frozen until they were shipped on dry ice to Nebraska for analysis.

**TABLE I. Details of Females and Fecal Sample Sizes Used in This Study**

<table>
<thead>
<tr>
<th>Female ID</th>
<th>Birthdate</th>
<th>Social status</th>
<th>Ovulatory status</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Sept 1981</td>
<td>Dominant</td>
<td>Ovulatory</td>
<td>20</td>
</tr>
<tr>
<td>291</td>
<td>13 Sept 1988</td>
<td>Dominant</td>
<td>Ovulatory</td>
<td>81</td>
</tr>
<tr>
<td>436</td>
<td>25 Nov 1991</td>
<td>Dominant until 10/29/98;</td>
<td>Ovulatory in both statuses</td>
<td>79</td>
</tr>
<tr>
<td>524</td>
<td>4 Oct 1993</td>
<td>Dominant</td>
<td>Ovulatory</td>
<td>40</td>
</tr>
<tr>
<td>539</td>
<td>15 Nov 1993</td>
<td>Dominant</td>
<td>Ovulatory</td>
<td>31</td>
</tr>
<tr>
<td>603</td>
<td>30 Sept 1994</td>
<td>Subordinate until 10/29/98;</td>
<td>Ovulatory in both statuses</td>
<td>67</td>
</tr>
<tr>
<td>629</td>
<td>1994</td>
<td>Dominant</td>
<td>Ovulatory</td>
<td>14</td>
</tr>
<tr>
<td>650</td>
<td>11 Sept 1995</td>
<td>Subordinate</td>
<td>Ovulatory</td>
<td>23</td>
</tr>
<tr>
<td>651</td>
<td>1993</td>
<td>Dominant</td>
<td>Ovulatory</td>
<td>53</td>
</tr>
<tr>
<td>672</td>
<td>1993</td>
<td>Dominant</td>
<td>Ovulatory</td>
<td>17</td>
</tr>
<tr>
<td>685</td>
<td>14 Aug 1996</td>
<td>Subordinate</td>
<td>Anovulatory</td>
<td>14</td>
</tr>
<tr>
<td>688</td>
<td>5 Nov 1996</td>
<td>Subordinate</td>
<td>Anovulatory</td>
<td>8</td>
</tr>
<tr>
<td>689</td>
<td>5 Nov 1996</td>
<td>Subordinate until 11/30/98, dominant after 1/19/99</td>
<td>Anovulatory, then oovulatory</td>
<td>37</td>
</tr>
<tr>
<td>703</td>
<td>1995</td>
<td>Dominant</td>
<td>Ovulatory</td>
<td>45</td>
</tr>
<tr>
<td>704</td>
<td>1994</td>
<td>Subordinate</td>
<td>Ovulatory</td>
<td>5</td>
</tr>
<tr>
<td>720</td>
<td>1994</td>
<td>Subordinate until 2/99, then dominant</td>
<td>Anovulatory, then oovulatory</td>
<td>51</td>
</tr>
<tr>
<td>721</td>
<td>1994</td>
<td>Dominant</td>
<td>Ovulatory</td>
<td>51</td>
</tr>
<tr>
<td>723</td>
<td>15 Nov 1997</td>
<td>Subordinate</td>
<td>Anovulatory</td>
<td>10</td>
</tr>
<tr>
<td>CM11</td>
<td>Nov 1992</td>
<td>Dominant</td>
<td>Ovulatory</td>
<td>23</td>
</tr>
<tr>
<td>CM13</td>
<td>Nov 1993</td>
<td>Dominant</td>
<td>Ovulatory</td>
<td>12</td>
</tr>
<tr>
<td>MA9</td>
<td>Oct 1995</td>
<td>Dominant</td>
<td>Ovulatory</td>
<td>10</td>
</tr>
<tr>
<td>ST6</td>
<td>Mar 1993</td>
<td>Dominant</td>
<td>Ovulatory</td>
<td>29</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>710</strong></td>
</tr>
</tbody>
</table>
We allowed the fecal samples to thaw at room temperature, and placed 0.125-g fractions of each sample in a round-bottom, 20-ml test tube. We removed seeds, leafy material, and insect parts from the fecal sample before it was weighed. We added a 5.0-ml volume of solubilizer (40% methanol: 60% phosphate-buffered saline (PBS)) and placed the tubes on a shaker rack overnight (12–16 hr). We pelleted the particulate matter in the resulting slurry by centrifugation for 15 min at 2,000 g, and separated the supernatant into a clean test tube. We refroze the extracted samples and stored them prior to assay.

Cortisol Assay

We measured fecal cortisol using a previously characterized assay (Smith & French, 1997a). We coated microtiter plates (Nunc Maxisorp F96) with rabbit anticortisol (R4866) diluted 1:12,000 in sodium bicarbonate coating buffer. We further diluted extracted fecal samples with assay buffer (1:10) prior to measurement to place the sample concentration within the range of the standard curve. We diluted cortisol standards (ICN Biomedical, Costa Mesa, CA) in a 1:10 dilution of extraction buffer. The cortisol standards ranged from 1,000 to 7.8 pg/well in halving concentrations. We diluted cortisol-horseradish peroxidase (HRP) conjugate (batch 11/94) in PBS and added it to the wells. After a 2-hr incubation, we washed the plate wells and added hydrogen peroxide and ABTS (2,2'-Azino-bis(3-thylbenzthylene-6-sulfonic acid)) as a chromogen. We measured absorbance 1 hr later at 410 nm (reference 570 nm) in a Dynatech MR5000 microplate reader, and calculated the sample concentrations using a four-parameter sigmoidal curve-fitting function. The intra- and inter-assay coefficients of variation for high and low concentrations of a fecal quality control pool were 6.7% and 13.6%, and 6.1% and 16.0%, respectively. Cortisol standards and serial dilutions of extracted feces collected from females at different times during pregnancy produced parallel displacement curves. We determined the accuracy of the assay by spiking the supernatant of a fecal sample with 100 pg of cortisol standard. The recovery of cortisol standard was 101% ± 2.0% (n=6).

Data Analysis

We classified the reproductive stages as nonpregnant (including cycling and noncycling samples) or first, second, or third trimester by counting backward 132 days from parturition [Wilson, 1977] and examining gonadal steroid levels. Most females are represented in more than one trimester (see Fig. 1 for sample size during each trimester). However, due to various factors, such as different dates of conception, the start of the field season, and the dates on which groups/females entered the study, not all females are represented in each trimester. Females that produced offspring during the study were also classified as primiparous (first parturition) or multiparous (second or later parturition). Because some of the data were neither normal nor transformable to normality, we used nonparametric Mann-Whitney U-tests [Sokal & Rohlf, 1981] in two-sample comparisons and Kruskal-Wallis tests to compare multiple groups. Because nonparametric statistics were used, all data are presented as medians and interquartile ranges.
RESULTS

Effects of Reproductive Status

Cortisol levels varied significantly according to reproductive stage (Kruskal-Wallis test, $\chi^2=30.27, P<0.0001$). When compared to nonpregnant (including cycling and noncycling) levels, cortisol levels were significantly lower during the first trimester of pregnancy (Mann-Whitney test, $U=55.0, P=0.006$; Fig. 1). The levels rose slightly but nonsignificantly in the second trimester compared to the first trimester (Mann-Whitney test, $U=65.0, P=0.103$), and then rose significantly during the third trimester of pregnancy (Mann-Whitney test, $U=115.0, P=0.0005$) compared to the second trimester. Primiparous females did not differ from multiparous females during the nonpregnant stage or the first two trimesters, but had significantly higher cortisol levels during the third trimester of pregnancy (multiparous females=10; primiparous females=6; Mann-Whitney test, $U=73.0, P<0.02$; Fig. 2).

Effects of Dominance Status

Four females had nonpregnant cortisol values as both dominants and subordinates. When these were compared in a paired-sample test, there was no significant difference in cortisol concentrations between statuses (Wilcoxon paired-sample test, NS; Fig. 3). When nonpregnant cortisol values were compared for dominant females, ovulatory subordinates, and anovulatory subordinates, no significant differences were found (Kruskal-Wallis test, $\chi^2=0.52, P=0.7694$; Fig. 4).
Cortisol levels in wild golden lion tamarin females appear to be primarily influenced by reproductive stage, as opposed to social status. There was significant variation in cortisol across pregnancy, with lower levels in the first trimester of pregnancy (compared to nonpregnant levels, which may include ovulatory peaks), and then a significant rise in the third trimester. In contrast, dominant and subordinate females did not differ in cortisol levels.

**DISCUSSION**

Cortisol levels in wild golden lion tamarin females appear to be primarily influenced by reproductive stage, as opposed to social status. There was significant variation in cortisol across pregnancy, with lower levels in the first trimester of pregnancy (compared to nonpregnant levels, which may include ovulatory peaks), and then a significant rise in the third trimester. In contrast, dominant and subordinate females did not differ in cortisol levels,
even when ovulatory subordinates were compared separately from anovulatory subordinates.

These patterns are generally consistent with data from several other callitrichid species, including common marmosets [Ziegler & Sousa, 2002], cotton-top tamarins [Ziegler et al., 1995], and Wied’s black tufted-ear marmosets [Smith & French, 1997b]. In addition, they fit the predictions of Abbott et al. [2003] based on the golden lion tamarin social system. Wild subordinate female golden lion tamarins have extensive social support within the group, and are subject to only low levels of harassment (culminating near the time of expulsion from the group). They also live in groups that consist of close kin [Baker et al., 1993; Dietz & Baker, 1993]. In the statistical models generated by Abbott et al. [2003], all of these features were associated with similar or lower levels of baseline cortisol titers in subordinates relative to dominants. Our results clearly indicate that fecal cortisol concentrations did not differ on the basis of social status (dominant vs. subordinate) or ovulatory status (anovulatory vs. ovulatory).

The elevated third trimester concentrations of cortisol observed in this study are consistent with results from other callitrichid primates [Smith & French, 1997b; Ziegler et al., 1995, 2004] and primates in other taxa, including human females [French et al., 2004; McLean & Smith, 1999]. The high late-pregnancy levels of glucocorticoids in primates are associated with elevated concentrations of circulating corticotropin-releasing hormone (CRH), which has a placental origin [Mastorakos & Ilias, 2003; Smith et al., 1999]. In the second half of gestation, there is increased steroidogenesis in the developing fetal adrenal gland (principally the synthesis and release of dehydroepiandrosterone sulfate) [Coulter & Jaffe, 1998]. Increased DHEAS and possibly other stimuli may contribute to the rise in placental CRH synthesis and release, and hence maternal

![Fig. 4. Nonpregnant cortisol levels (ng/g feces) for females according to dominance and reproductive status (median ± interquartile range).](image-url)
hypercortisolism, since removal of the fetus (but not the placenta) in pregnant females leads to lower levels of maternal ACTH, cortisol, and DHEAS [Umezaki et al., 2001]. We previously reported [Bales et al., 2002] that female lion tamarins that give birth to larger infants have higher rises in cortisol than females that give birth to smaller infants. This difference may reflect differences in fetal maturation and hence differences in activity in the fetal adrenal glands.

Our finding in the present study that primiparous females have higher cortisol levels in the third trimester of pregnancy is interesting in the light of previous results that subordinate (usually primiparous) mothers had higher rises in estrogen conjugates during pregnancy [French et al., 2003]. To the extent that placental steroidogenesis in lion tamarins is similar to that in other primates, this result is consistent with the observation that adrenal steroids appear to be critical precursors or prohormones for placental estrogens [Novy & Walsh, 1983; Waddell et al., 1992]. Elevated glucocorticoids, therefore, would provide more substrate for the biosynthesis of estrogens. Regardless of their origins, higher cortisol and estrogen levels in first-time mothers may contribute to enhanced maternal behavior and responsiveness to infant stimuli [Bahr et al., 1998; Fleming et al., 1997; Krpan et al., 2005; Leon, 1992; Pryce et al., 1988].

While all captive subordinate golden lion tamarins studied have been ovulatory, some wild subordinate golden lion tamarins have shown evidence of anovulation [French et al., 2003]. However, the absence of differences in cortisol levels suggests that, as in captive marmosets [Saltzman et al., 1994, 2000], this reproductive suppression is not caused by elevated cortisol levels. Further studies of free-ranging females, especially dispersing individuals seeking breeding opportunities, will be useful for disentangling reproductive and social effects.

**ACKNOWLEDGMENTS**

K. Bales was supported by predoctoral fellowships from the NSF and the University of Maryland, as well as Sigma Xi, Friends of the National Zoo, and a Biology of Small Populations Research training grant. Research at Poço das Antas is supported by NSF grants SBR-9828687 and BCS-0216096 to J. Dietz, A. Baker, and J. French. We thank CNPq, IBAMA, and the Associação Mico-Leão-Dourado for permission to conduct this research. We thank J. Mickelberg, D. Vogt, R. Lake, A. Martins, and the Ecologia field team for research assistance. This research complies with the guidelines of the University of Maryland Animal Care and Use Committee, and all relevant Brazilian laws.

**REFERENCES**


Cortisol in Female Tamarins / 35