

Parent–Daughter Relationships and Social Controls on Fertility in Female Common Marmosets, *Callithrix jacchus*

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This study examined the reproductive status of mothers and daughters to determine if parent–eldest daughter interactions would influence the daughter's fertility in the cooperatively breeding common marmoset, *Callithrix jacchus*. Twice weekly fecal samples were collected for hormonal analyses from seven daughters and their mothers and analyzed for cortisol, estradiol, and progesterone by EIA. Behavioral data were collected three times weekly while the daughters were under three conditions: natal family, after removing from the family and pairing with a novel male, and after removing from the paired condition and placed back with the family (renatal). Under the natal condition, five of the seven daughters exhibited ovarian cycles while their mother was pregnant or displaying ovarian cycling. The five cycling daughters spent significantly more time initiating affiliative behaviors with their mothers than with their fathers and showed significant changes in their behavior over the length of the ovarian cycle. However, aggression, submission, and sexual behaviors were very low for daughters in the natal phase. No differences between cortisol levels were found for a daughter and her mother. Cortisol levels showed a significant and sustained increase upon pairing within the first 20 days. All females but one cycled while paired. Six of the seven females were accepted back into the family after pairing and five of the seven females were ovulating. These results suggest that marmoset daughters are not necessarily reproductively suppressed while living with the family. Additionally, these data suggest that female marmosets that leave their family to explore mating opportunities with other groups may return to their family without repro-

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Among primates, the callitrichid monkeys (family Callitrichidae) may have the highest reproductive rate. Twin births are normal both in captivity and in the wild and females are capable of conceiving again 10 to 30 days following birth (McNeilly Abbott, Lunn, Chambers, and Hearn, 1981; Ziegler, Widowski, Larson, and Snowdon, 1990). However, reproductive potential maybe reached in only a few mature individuals per social group. Under most conditions in the wild and in captivity, all genera display levels of fertility control in daughter or subordinate females when they remain with a family past puberty, resulting in only one breeding female (Abbott, 1984; Saltzman, Severin, Schultz-Darken, and Abbott, 1997; Albuquerque, Sousa, Santos, and Ziegler, 2001). Mechanisms used to restrict breeding have mainly focused on endocrine and behavior inhibition (French, 1997). No suppression of ovarian cyclicity has been reported for daughter captive golden-lion tamarins (*Leontopithecus rosalia*) under family conditions (French and Sibley, 1987). *Saguinus* species have a complete suppression of ovulation found in daughters of captive intact family groups (Epple and Katz, 1984; Ziegler, Savage, Scheffler, and Snowdon, 1987a). However, in wild cotton-top tamarins a subordinate female has shown ovarian cycling and pregnancy when an unrelated male joined the group (Savage, Shideler, Soto, Causado, Giraldo, Lasley, and Snowdon, 1997).

The greatest flexibility of response to fertility control appears to occur in *Callithrix* species. In captivity some

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families are associated with complete fertility suppression in daughters, while in other families, many eldest daughters will display ovarian cycles (for pygmy marmosets, *Cebuella pygmaea*, Carlson, Ziegler, and Snowdon, 1997; for common marmosets, *Callithrix jacchus*, Abbott, 1984; Hubrecht, 1989; Saltzman *et al.*, 1997; for Wied's black tufted-ear, *Callithrix kuhli*, Smith, Schaffner, and French, 1997). Thus, marmosets may provide the best model for determining the mechanisms of fertility suppression. Field studies on wild common marmosets have reported variability in the breeding systems (Digby and Ferrari, 1994; Digby, 1995) but only one study had sufficient hormonal data to indicate fertility status (Albuquerque *et al.*, 2001). However, several studies provide evidence breeding may be restricted to only one mature female per group or that two females can be pregnant in the same group (Scanlon, Chalmers, and Monteiro da Cruz, 1988; Digby and Ferrari, 1994; Mendes Pontes, and Monteiro da Cruz, 1995).

In captivity, when female marmosets and cotton-top tamarins, *Saguinus oedipus*, are raised with their natal family they can remain there well beyond puberty and participate in infant care of younger siblings. There appear to be factors of chemical and behavioral cues involved in the fertility inhibition seen in callitrichid monkeys (Barrett, Abbott, and George, 1990; Epple and Katz, 1984; Savage, Ziegler, and Snowdon, 1988). Additionally, evidence indicates that the cause of fertility suppression in subordinate marmosets and tamarins does not appear to be caused by social stress as exhibited by elevated cortisol levels.

In the cotton-top tamarin, the common marmoset, and the Wied's marmoset, females which do not display ovarian cycling when in a subordinate status do not show elevated levels of cortisol indicative of stress (Abbott, McNeilly, Lunn, Hulme, and Burden, 1981; Saltzman, Schultz-Darkin, Scheffler, Wegner, and Abbott, 1994; Smith and French, 1997; Ziegler, Scheffler, and Snowdon, 1995). Subordinate female common marmosets in social groups of unrelated individuals have significantly lower levels of plasma cortisol than the dominant cycling female (Saltzman *et al.*, 1994). Noncycling daughter female cotton-top tamarins display basal urinary cortisol levels that are no different than the levels seen in female tamarins which have been cycling long term, but are significantly lower than cortisol levels seen in females that combine ovarian cycling with removal from the group (Ziegler *et al.*, 1995). Urinary cortisol concentrations elevate significantly during social transitions in the female cotton-top tamarin when females are removed from the fam-

ily. Urinary cortisol profiles in Wied's marmosets are consistent with those of the cotton-top tamarins showing cortisol levels to be the same for daughters living natal as adult females and that cortisol elevates during social transition (Smith and French, 1997). Female common marmosets show that circulating cortisol levels are responsive to ovarian cycling (Saltzman *et al.*, 1994) and cortisol levels do not appear to predict social status or increase in response to encountering strange conspecifics (Saltzman *et al.*, 1997). Cortisol levels in postpubertal cycling and noncycling daughter common marmosets living in related families have not yet been reported.

Based on field and captive studies, female common marmosets have four possible strategies available for ensuring breeding opportunity: (1) daughters can remain in the natal family waiting for the opportunity to obtain breeding status; (2) daughters can emigrate to other groups where it may be possible for more than one female to breed; (3) daughters could form new groups where they may become the sole breeding female; or (4) daughters may have intergroup matings while remaining in the natal group. In captivity, common marmosets show little aggressive interaction with their mothers even when undergoing ovarian cyclicity or after matings with an unrelated male (Hubrecht, 1989; Saltzman *et al.*, 1997). In the wild, aggressive interactions between dominant and subordinate females also appear to be rare (Digby, 1995; Lazaro-Perea, Snowdon, and Santee, 1997). High levels of group transfers have been reported for some groups of common marmosets (Hubrecht, 1985; Scanlon *et al.*, 1988; Mendes Pontes and Monteiro da Cruz, 1995; Lazaro-Perea, 2000). Hubrecht (1985) also reports on a female common marmoset that was observed mating with an extragroup male on several occasions while living with the home group. Lazaro-Perea (2000) reports that at Nisia Floresta field station in northeastern Brazil, individual males and females leave their groups for interactions with members of the opposite sex from other groups and return within the same day. If sexually mature daughters can leave their family group for sexual encounters and then return, it becomes important to examine the response of the cooperative breeding family to sexually active daughters.

No external signs of ovulation can be detected in common marmosets. Without endocrine data, it will be difficult to assess the ovarian status of daughters when they emigrate or leave the group for intergroup encounters. Without these data, one can only speculate how fertility suppression of common marmosets

TABLE 1
Timing of Social Conditions and Reproductive State of Daughters and Mothers during the Natal Phase and Other Phases

Daughter No.	Age (months)	Reproductive condition and days in each social condition					Mother No.	Natal	Age (months)
		Natal	Paired	Renatal	No. of siblings				
386	23	Ovarian cycling (91)	Ovarian cycling (58)	Ovarian cycling (32)	4	216	Pregnant (early)	64	
388	23	Ovarian cycling (61)	Ovarian cycling (81)	Ovarian cycling (36)	5	216	Pregnant (mid-end)	64	
410	18	Ovarian cycling (71)	Ovarian cycling (63)	Ovarian cycling (10)	4	216	Pregnant (mid-end)	64	
402	20	Ovarian cycling (57)	Ovarian cycling (56)	Ovarian cycling (62)	2	80	Conception and early pregnant	78	
416	18	Ovarian cycling (61)	Ovarian cycling (70)	Noncycling (57)	2	65	Ovarian cycling	80	
418	18	Noncycling (49)	Noncycling (64)	Noncycling (44)	1	138	Ovarian cycling	62	
430	12	Noncycling (58)	Ovarian cycling (66)	Ovarian cycling (43)	1	31	Noncycling	96	

occurs in the wild or if the condition is mainly a result of captivity, where females are in closer contact with one another, allowing chemical signaling to be more effective.

The goals of this study were to determine (1) the reliability of using fecal steroids for documenting adrenal and ovarian changes; (2) the interaction of fecal cortisol with ovarian functioning in reproductively aged captive daughter marmosets living with their family; (3) the effect of social transition on cortisol levels; and (4) the acceptability of daughter marmosets returning to their families after breeding with a novel male. The proposed studies on captive marmosets simulate the strategies postpubertal daughters may use to establish a position in a group that ultimately would provide breeding status or transfer back into their natal group if immigration proves unsuccessful. The steroid data obtained in this study under the different social conditions will provide us with a framework with which to interpret steroid levels in fecal samples collected from wild marmosets.

MATERIALS AND METHODS

Subjects and Housing

Seven daughters, 12 to 23 months of age, and their families were chosen as subjects (Table 1). All the subjects lived in separate families except for three of the females. These three were all postpubertal and studied sequentially but with some overlap in fecal and behavioral collection. All females had been living in their intact family groups from birth and had been born at the Wisconsin Regional Primate Research Center (WRPRC). Their mothers ranged in age from 62 to

96 months. Cages for the marmoset families ranged in size from $2.2 \times 3.1 \times 2.8$ m for the largest family, consisting of eight individuals (1 month to 5 years of age), to $0.6 \times 0.91 \times 0.183$ m for families consisting of six or fewer individuals. All daughters used as study subjects had a twin, either a male or female, and all but two had younger siblings. Dietary and husbandry conditions for the marmoset colony have been described by Saltzman *et al.* (1997) but modified with the addition of fresh fruit and nuts in the morning to the cages of study subjects. Lighting consisted of a 12/12 light/dark cycle and humidity was kept at approximately 50%. All housing conditions and social manipulations were preapproved by the Animal Care and Use Committee at the University of Wisconsin.

Social Conditions

Daughters were examined under three conditions: intact natal families (Natal Condition), paired with an unrelated male (Paired Condition), or reinstated in the natal families (Renatal Condition). Daughters and their behavioral interactions with their mother and father were studied under the Natal Condition for approximately 60 days (range, 49–91). Daughters were then removed from their families and placed in cages in other parts of the colony that shared no auditory, visual, or olfactory communication with their families. An unrelated male of approximately the same age (range, 19–35 months) was then placed in the cage with the female. Behavioral and hormonal data were collected on the daughters during this stage for approximately 60 days (range, 58–81) before removing the females and placing them back in their original families. To terminate any conceptions, fe-

males were administered with a prostaglandin F_{2α} analogue while living paired with a mate or prior to returning to their families. All females had younger siblings in the family when they were returned to the family except two females, who had twin brothers still in the family. Behavioral interactions and hormone samples were collected for approximately 45 days (range, 32–62) under the last condition.

Data Collection

Behavior. Instantaneous focal animal recordings of behavior were taken every 15 s for 30 min three times per week to study the behavior of daughter marmosets toward their mother and father as well as toward their mate when paired. Timings of behavioral observations were picked each week to represent both morning and afternoon. Behavior was recorded on a laptop computer (Tandy 100) and then downloaded to a VAX mainframe computer. The behavioral categories were modified from Saltzman *et al.* (1997) and are listed in Table 1. Behaviors were scored under the following categories: Affiliative, Submission, Courtship and sex, Investigative behaviors, Aggression, and Individual behaviors. Individual behaviors under these categories are listed in Table 2. Behaviors were recorded for frequencies as initiated and received and some behaviors were recorded for duration (grooms, huddles). Four different observers collected the data from the seven females. Reliability checks between observers were taken at the beginning of the study. Reliability was found to be 90.15% ($n = 4$).

Fecal samples. For each female and her mother under the Natal Condition, fecal samples were collected on average two times per week. All samples were collected in the afternoon hours (between 1 and 4 PM) to reduce diurnal variation contribution to hormonal concentration for progesterone and cortisol (Sousa and Ziegler, 1998). Fecal samples were collected only on the daughter when she was under the Paired Condition or the Renatal Condition. To collect the feces, a clean paper cloth was placed at the bottom of the cage to cover the absorbent litter. For females in the large cages, an observer watched the female of interest until she defecated and then entered the cage and collected the sample from the litter. Samples were picked up after entering the cage and the fecal pellet was stored in a glass tube. The samples were capped and stored immediately on ice if the collector was remaining in the room or directly in a freezer at -20°C until hormonal analyses.

TABLE 2

Sociosexual Behaviors of *Callithrix jacchus*

Affiliative	Aggression
Initiated or received	Initiated or received
Proximity	Vocal: erh-erh
Huddle	Ear tufts flick
Groom	Frown
Autogroom	Cuff
Submission	Chase
Vocal: nga-nga	Fight
Facial submit	Attack
Continuous submit	Snap bite
Avoid	Individual behaviors
Courtship and sex	Sexual
Initiated or received	Arch bristle
Sniff fur	Scent mark
Tongue flick	Genital present
Mount	Activity
Attempt to mount	Alone/stationary
Active refusal	Alone/moving
Arch bristle strut	In nestbox
Follow	
Investigative behaviors	
Initiated or received	
Sniff scent mark	
Sniff substrate	
Anogenital inspection	

Hormonal Analyses

All fecal samples were processed according to methods reported by Sousa and Ziegler (1998). Briefly, 0.1 g of feces was weighed and extracted into 5 ml of ethanol/water. A portion, 500 μl , was taken for solvolysis to remove the excessive conjugation of estradiol found in marmoset feces by acid solvolysis and ethyl acetate extraction (Ziegler, Scheffler, Wittwer, Schultz-Darken, Snowdon, and Abbott, 1996). Samples were resuspended in 500 μl of ethanol and stored until assay quantification. Progesterone, estradiol, and cortisol assays were all performed on the purified sample. Column chromatography separation of steroids was not used since comparisons of daily steroid levels from one female over an entire ovarian cycle measured with and without celite chromatography showed the same profiles. The only difference was that in the nonchromatographed technique both progesterone and estradiol showed a greater magnitude of change in steroid concentration between the follicular and luteal phases. The two techniques showed correlation coefficients of $r^2 = 0.73$, $y = 3.23x + 2485.92$, $P < 0.0001$, for estradiol and $r^2 = 0.68$, $y = 1.11x + 131.13$, $P < 0.0001$, for progesterone.

All samples were analyzed by EIA techniques for

steroid measurement. Progesterone, estradiol, and cortisol methods and validations have been reported by Sousa and Ziegler (1998). For all steroid determinations, samples were run in duplicate with progesterone at 50 μl , estradiol at 10 μl , and cortisol at 100 μl of a 1:50 dilution. Progesterone CVs were 7.6%, 3.6% for intra-assay and 24.3%, 26.5% for interassay variations of low and high pools, respectively ($n = 19$ assays). Estradiol CVs were 6.3%, 6.2% for intra-assay and 16.9%, 11.5% for interassay variation of low and high pools, respectively ($n = 18$ assays). Cortisol CVs were 3.9%, 3.8% for intra-assay and 16.8%, 16.0% for interassay variation of low and high pools, respectively ($n = 20$ assays).

Determination of Ovarian Cycles and Pregnancies

Concentrations of reproductive hormones, estradiol and progesterone, were graphed to determine the reproductive condition of mothers and daughters. Ovarian cycles could be determined based on the cyclical increases of progesterone and estradiol that remained elevated for at least 10 days and were preceded by baseline levels for at least 5 days. Ovulation was assumed to have occurred when progesterone and estradiol levels were 1.5 times the mean of the preceding baseline values, as has been suggested by Harlow, Hearn, and Hodges (1984) for plasma levels. In marmoset feces, both estradiol and progesterone follow similar profiles, with a sustained increase in estradiol following an onset of progesterone increase indicating the onset of the luteal phase. The onset of a sustained increase in progesterone and estradiol occurs 2–4 days following the plasma LH peak (Ziegler *et al.*, 1996). Since LH is not measurable in the feces due to degradation of protein molecules, no determination of ovulation was possible. However, the periovulatory period could be estimated roughly based on the timing of the increase in progesterone and estradiol and the knowledge that ovarian cycles are an average of 30 days in the marmoset (Harding, Hulme, Lunn, Henderson, and Aitken, 1982). Pregnancy was defined based on fecal hormonal profiles and backcounting from parturition to the previous onset of sustained increase in estradiol and progesterone using a gestational length of 148 days (Chambers and Hearn, 1979).

One ovarian cycle per social condition was divided into phases for each daughter for the purpose of examining the behavioral profiles across the ovarian cycle. Follicular and luteal phases were divided according to the criteria reported by Harlow, Gems, Hodges, and Hearn (1983). The timing of the sustained

increase in fecal progesterone was taken as an indication that ovulation had occurred. The day of the progesterone increase plus the 3 days previous were estimated to be the periovulatory phase of the cycle. The 6 days prior to this were considered the follicular phase of the cycle and the 18 days following the periovulatory phase were considered the luteal phase of the cycle. This provides for a 28-day cycle, with the ovulatory phase occurring around the middle of the cycle (between 6 days of the follicular phase and 18 days of the luteal).

Statistical Analysis

CVs for each steroid were determined using equations reported by Robard (1974). Comparisons between different assay systems used Pearson correlation. The χ^2 test of independence was used to determine if daughter's reproductive condition was dependent on mother's reproductive condition. Differences between mothers and daughters in cortisol levels were compared by paired *t* test. Cortisol data were not normally distributed under the different social conditions and therefore, nonparametric tests were used. To determine the effect of social condition on cortisol, Friedman and Wilcoxon tests were used to assess changes in cortisol levels across the three social phases. Twenty days pre- and postmove were averaged for each female and tested with the Wilcoxon signed ranks test. The Wilcoxon test was also used to compare time spent in different behaviors directed toward mothers and fathers or received from mothers and fathers. Mean frequencies of behaviors during different phases of the ovarian cycle were compared by Friedman two-way analysis of variance.

RESULTS

Natal Condition: Reproductive State

Five of the seven daughters (71%) showed ovarian cycling while living with the family (Table 3). For four of these females, their mother was pregnant during the sampling period and another female had a mother who showed ovarian cycling. Two daughters showed no signs of ovarian cycling during the 2 months, while one had a cycling mother and the other did not. Cycling or pregnancy hormonal profiles for both mothers and daughters were identifying by luteal levels of progesterone over 200 ng/g of feces and estradiol over 2 $\mu\text{g/g}$ of feces (Fig. 1). Four of the females showed

TABLE 3
Frequency of Mother–Daughter and Father–Daughter Behavioral Interactions under the Natal and Renatal Conditions

	Natal		Renatal	
	Cycling daughters	All daughters	Cycling daughters	All daughters
Affiliative				
Mother initiates	0.16 ± 0.05	0.11 ± 0.05	0.22 ± 0.05	0.16 ± 0.10
Daughter initiates	10.05 ± 5.46	7.91 ± 4.02	9.06 ± 3.06	6.91 ± 4.08
Father initiates	0.44 ± 0.32	0.32 ± 0.23	0.29 ± 0.52	0.19 ± 0.43
Daughter initiates	5.55 ± 3.73	4.88 ± 2.62	5.74 ± 5.26	4.13 ± 4.81
Aggression				
From mother	0.03 ± 0.03	0.024 ± 0.02	0	0.10 ± 0.23
Toward mother	0	0.007 ± 0.007	0	0
From father	0.006 ± 0.006	0.019 ± 0.014	0	0.03 ± 0.07
Toward father	0	0.023 ± 0.016	0	0.01 ± 0.02
Submission				
Toward mother	0	0.027 ± 0.02	0	0.20 ± 0.05
Toward father	0.01 ± .01	0.11 ± 0.06	0.17 ± 0.25	0.13 ± 0.20
Sexual				
Received from mother	0	0	0	0
Toward mother	0.07 ± 0.07	0.05 ± 0.05	0	0
Received from father	0	0	0	0.02 ± 0.05
Toward father	0.134 ± 0.134	0.096 ± 0.1	0	0
Investigative				
Toward mother	0.08 ± .06	0.06 ± 0.05	0.01 ± 0.03	0.01 ± 0.02
From mother	0	0.007 ± 0.007	0	0
Toward father	0.02 ± 0.02	0.014 ± 0.014	0	0.01 ± 0.05
From father	0.12 ± 0.07	0.09 ± 0.05	0.02 ± 0.05	0.02 ± 0.04
Activity of daughter				
Moving	17.49 ± 10.39	23.30 ± 9.55	16.0 ± 5.42	18.42 ± 4.85
Stationary	35.72 ± 13.66	35.62 ± 12.18	54.11 ± 14.5	55.64 ± 11.49

Note. Data are expressed as means ± SEM. $N = 5$ for cycling daughters and $N = 7$ for all daughters.

consistent ovarian cycles with the luteal concentration and duration of approximately 20 days, indicating apparently normal cycles, while one female had only one normal cycle during the 60 days of sampling.

Reproductive activity of the daughters was not influenced by the reproductive activity of other females in the family. A χ^2 analysis indicated independence between the reproductive condition of the mother and the reproductive condition of the daughter ($\chi^2 = 3.84$, $df = 1$, $P = 0.05$). The three daughters studied from the same family all showed ovarian cycles during the natal phase. Overlapping sample collection indicated ovarian cycling in two of the daughters at the same time. Age may have been a factor in the onset of ovarian activity since the only noncycling daughters were two of the youngest: 12 and 18 months.

Averaged cortisol levels during the entire natal period are shown for mothers and daughters based on their reproductive condition in Fig. 2. Daughters, whether cycling or noncycling, had higher mean levels of fecal cortisol under the Natal Condition (271.75

ng/g ± 70.64) than mean cortisol levels in mothers for all reproductive states (164.40 ng/g ± 41.59) and this neared significance ($t = 2.23$, $df = 6$, $P = 0.067$). However, in an individual comparison between mother and daughter, a daughter's mean cortisol level for the natal phase was not significantly different from her mother's. Mothers who were pregnant had cortisol levels that changed from basal levels (<100 ng/g of feces) during the first trimester of pregnancy to very high levels in the last month before parturition (200–1500 ng/g).

Natal Condition: Behavior

Mean frequency of interactions between daughters and parents indicated that daughters initiated more interactions with their mothers than mothers initiated with their eldest daughters ($Z = -2.37$, $P = 0.02$, $n = 7$) (Table 3). Eldest daughters also initiated more affiliate behavior with their fathers than fathers did with their eldest daughters ($Z = -2.37$, $P = 0.02$, $n = 7$).

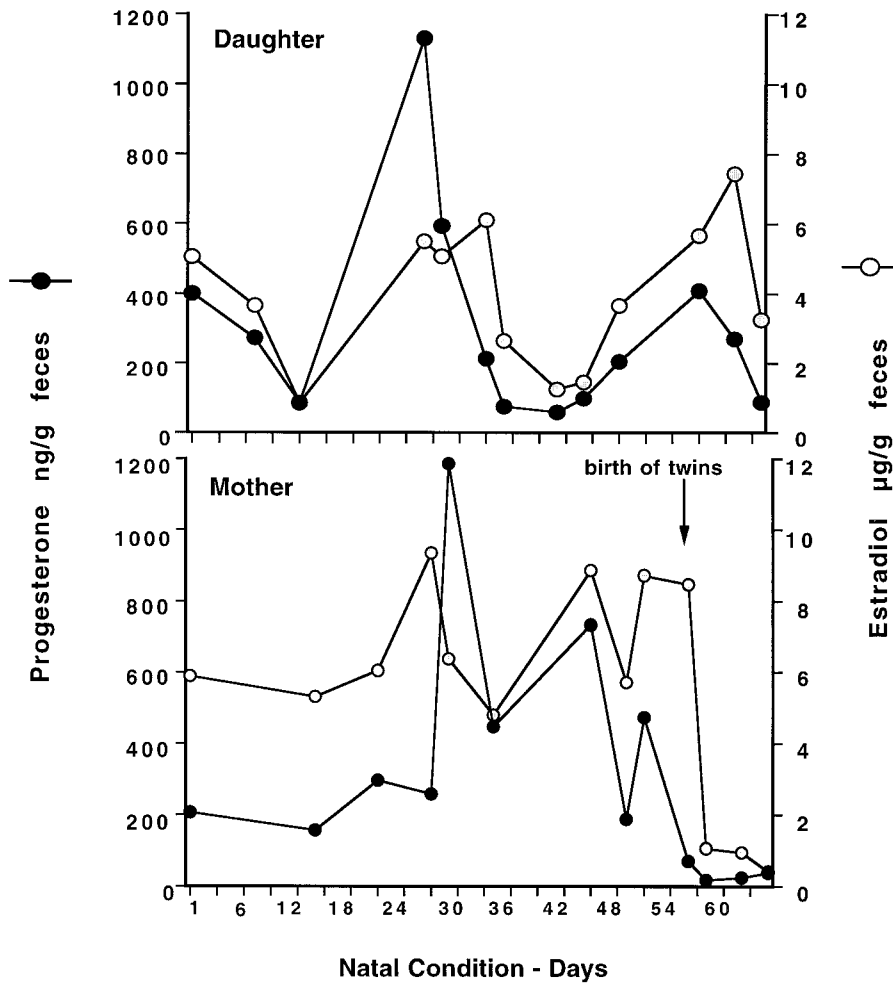


FIG. 1. Profiles of fecal progesterone and estradiol concentrations for an 18-month-old daughter common marmoset displaying ovarian cycling and her pregnant mother during the last trimester. Sampling for both females occurred at the same time.

Mothers and fathers initiated very few affiliative interactions with their cycling eldest daughters and essentially no interactions with their eldest noncycling daughters. Cycling daughters initiated significantly more of their affiliate time with their mothers than with their fathers ($Z = -2.02$, $P = 0.04$, $n = 5$). Daughters did not initiate aggressive behaviors with their mothers or fathers. Aggressive interactions initiated by mothers or fathers were extremely low, with only one mother showing an aggressive act toward a daughter and only two fathers showing an aggressive interaction with an eldest daughter. Sexual behaviors were extremely low whether the daughter was cycling or not. No chemical investigative behaviors were seen from mothers. The rate of fathers investigating daughters was low but was seen for three of the five cycling daughters. No investigations of noncycling daughters

was found. Activity time did not differ between the cycling daughters and all daughters.

Daughters showed significant changes in their behaviors associated with cycle phase, while their mothers' and fathers' behaviors toward the daughters did not change. Daughters showing ovarian cycles had significantly more initiations of affiliate contact with fathers during the luteal phase of their cycle (follicular, 0.64; periovulatory, 1.86; luteal, 2.63, $\chi^2 = 6.4$, $P = 0.04$) but not significantly with mothers. Scent marking in the daughters occurred at higher frequencies during the periovulatory phase of their cycle (follicular, 0.29; periovulatory, 0.6; luteal, 0.45; $\chi^2 = 7.6$, $P = 0.02$) as did spending time alone and staying still (follicular, 3.3; periovulatory, 8.93; luteal, 5.73; $\chi^2 = 7.6$, $P = 0.02$). Mothers' initiation of affiliative behaviors with daughters was too infrequent to test.

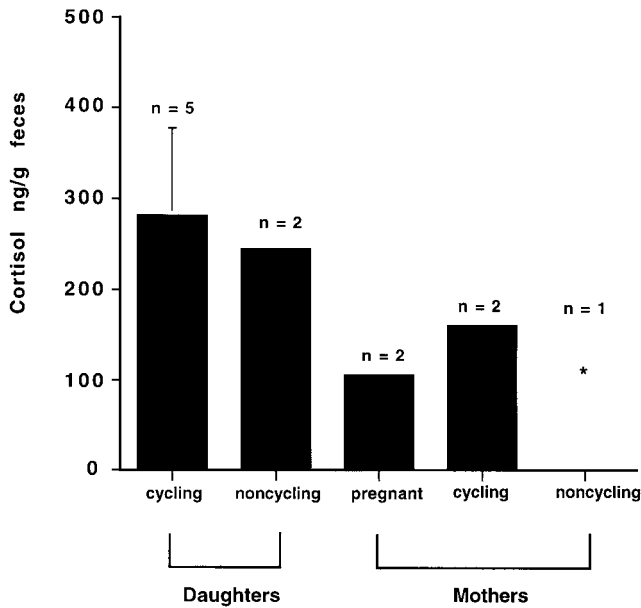


FIG. 2. Mean fecal cortisol concentrations under the natal condition for daughters who were cycling and noncycling and their mothers under three reproductive conditions.

Paired Condition: Reproductive State

The five cycling females continued to show ovarian cyclicity in the paired condition (see Table 1). One of the noncycling females showed an immediate onset of increased estradiol and progesterone levels indicating an ovarian cycle. One female did not show an ovarian response to being paired with a novel male. Two of the females became pregnant as determined by a long luteal phase (approximately 30 days) and these were terminated by the use of a prostaglandin $F2\alpha$ analogue.

Mean fecal steroid levels are shown for the different conditions in Fig. 3. Mean cortisol levels were different between the three conditions ($F_r = 8.86$, $n = 7$, $P = 0.01$), with mean cortisol levels significantly higher in the paired phase ($Z = 2.37$, $P = 0.02$) than the natal phase and the paired phase cortisol was significantly higher than the renatal phase ($Z = -2.19$, $P = 0.03$). The highest levels of cortisol were found in the first 20 days following transfer to the paired condition (mean cortisol levels of first 20 days, 760.60 ± 213.96 ng/g). These levels were significantly higher than cortisol levels found for the last 20 days in the natal condition (mean cortisol levels, 271.75 ± 70.64 ng/g, $Z = 2.37$, $n = 7$, $P = 0.02$). After the first 20 days for each female, cortisol levels dropped almost to levels seen for eldest daughters while in their natal groups. Mean

progesterone and estradiol levels did not change with the different social conditions.

Paired Condition: Behavior

Females showed behavioral responses to their change in housing conditions. Daughters spent more time alone and stationary in the paired phase (60.76 ± 5.4) than in the natal phase (35.62 ± 12.2) and this was almost significant ($Z = 1.86$, $P = 0.06$). Females spent more time initiating affiliate behaviors with males (15.9 ± 1.5) than males initiated toward females (0.91 ± 0.33) ($Z = -1.2$, $P = 0.03$). Sexual behaviors were frequent. Males mounted all females and attempted mounts were seen for all females except one. Even the one female who failed to exhibit ovarian cycling during the paired phase was mounted by her male. Tongue flicks were seen only in the paired phase. Other sexual behaviors, such as arch bristle, scent marking, and genital presenting by the female, were not significantly different in frequency under the natal and the paired conditions.

Renatal Condition: Reproductive State

Females were returned to their natal families after 2 months of being paired with a novel male. Mothers showed no overt aggression for six of the seven returned daughters. However, one mother repeatedly

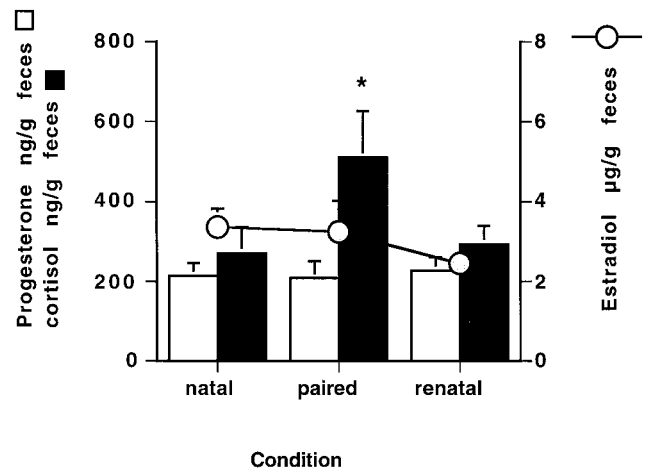


FIG. 3. Mean fecal cortisol, progesterone, and estradiol levels for seven daughter common marmosets under three conditions: natal, paired, and renatal. Mean cortisol under the paired condition was significantly higher than that under the other conditions ($F_r = 8.86$, $n = 7$, $P = 0.01$)

attacked her daughter after the return and the daughter (female 418) had to be removed from the family cage on the day of reintroduction and placed in the closet cage to the family. No other mother showed aggression toward a reintroduced daughter. The only other condition where aggression occurred during reintroduction was due to a younger female sibling.

Cortisol levels rose again in the females following reintroduction to their natal family but not as high as those when paired with a male. Cortisol levels were higher in the paired phase than the renatal phase ($Z = -2.20$, $n = 7$, $P = 0.03$) and the mean cortisol levels during the first 20 days of the renatal condition were not significantly different from those of the last 20 days of the paired condition. Three of the females responded with the highest and longest elevation of cortisol following the first move away from the natal family into a new cage with a novel male, while two females actually had a higher cortisol elevation following the move back to their natal family and two females showed the same elevated cortisol response to both social changes.

All the females who were cycling while paired with a novel male continued to cycle when reintroduced to their natal family, except one female (416). The female who was attacked by her mother during the renatal phase had not shown ovarian cycling under any of the conditions and continued not to display ovarian cycling. Figures 4A and 4B demonstrate the hormonal profiles of two female marmosets under the three social conditions.

Renatal Condition: Behavior

For five of the seven daughters, returning to the natal cage elicited no unusual behaviors from their parents. Daughters went back to their families without any agonistic behaviors, except for one female whose mother was aggressive and one female whose younger sister was aggressive. Comparing the behaviors of daughters between the natal and the renatal conditions indicated no statistical differences between the frequencies of family interactions (Table 3). Additionally, no statistical differences were found between cycling females or all females as to family interactions during the two phases. Although the frequencies of affiliative as well as aggressive behaviors were not significantly different under either condition, daughters actually initiated affiliative behaviors slightly less towards their mothers and received less affiliation from their father under the renatal condition.

DISCUSSION

This study found little evidence of reproductive suppression in common marmoset daughters while living in the natal family. Age was more of a factor for determining whether a female showed ovulatory cycles than was ovulatory suppression due to living with a mother as the dominant breeder or living with other female siblings. All three postpubertal females in one family showed ovarian cycles, but whether all three were cycling at one time is not known. Thus, while reproductive suppression has been reported under different living conditions at this colony (Saltzman *et al.*, 2000, 1998), in this sample, most daughters were cycling. Additionally, the daughter's reproductive condition also was independent of the mother's reproductive condition, i.e., whether a mother was pregnant or cycling did not influence the reproductive condition of the daughter. This indicates that under these conditions, mothers did not influence a reduction of ovarian function in their daughters.

Cortisol levels were responsive to ovarian activity. Although mean cortisol levels were slightly higher for daughters than for mothers under the natal condition, a direct comparison of mother and daughter during the same time period did not indicate a difference in cortisol levels. Mothers and daughters were not always in the same reproductive condition since two mothers were pregnant while we were monitoring their four daughters. There was no evidence of suppression of cortisol in socially subordinate daughters and this confirms data from Wied's marmosets and cotton-top tamarins (Smith and French, 1997; Ziegler *et al.*, 1995). However, cortisol levels are known to be responsive to female reproductive condition. In primates, exogenous estrogen treatment causes cortisol elevation (humans, Doe, Zinneman, Flink, and Ulstrom, 1960; Altemus, Roca, Galliven, Romanos, and Deuster, 2001; baboons, Pepe, Johnson, and Albrecht, 1982; squirrel monkeys, Coe, Murai, Wiener, Levine, and Siiteri, 1986) and periovulatory elevations of cortisol have been found in several species of primates, including marmosets (humans, Genazzani, Lemarchand-Beraud, Aubert, and Felber, 1975; tamarins, Ziegler *et al.*, 1995; marmosets, Saltzman *et al.*, 1997). Since most daughters in this study were cycling, we would expect to see cortisol levels similar to those of cycling mothers.

Fecal cortisol clearly responded to social changes for the daughter marmosets. For each female there was a significant sustained increase in cortisol levels for the first 2 weeks following pairing. Whether a female was

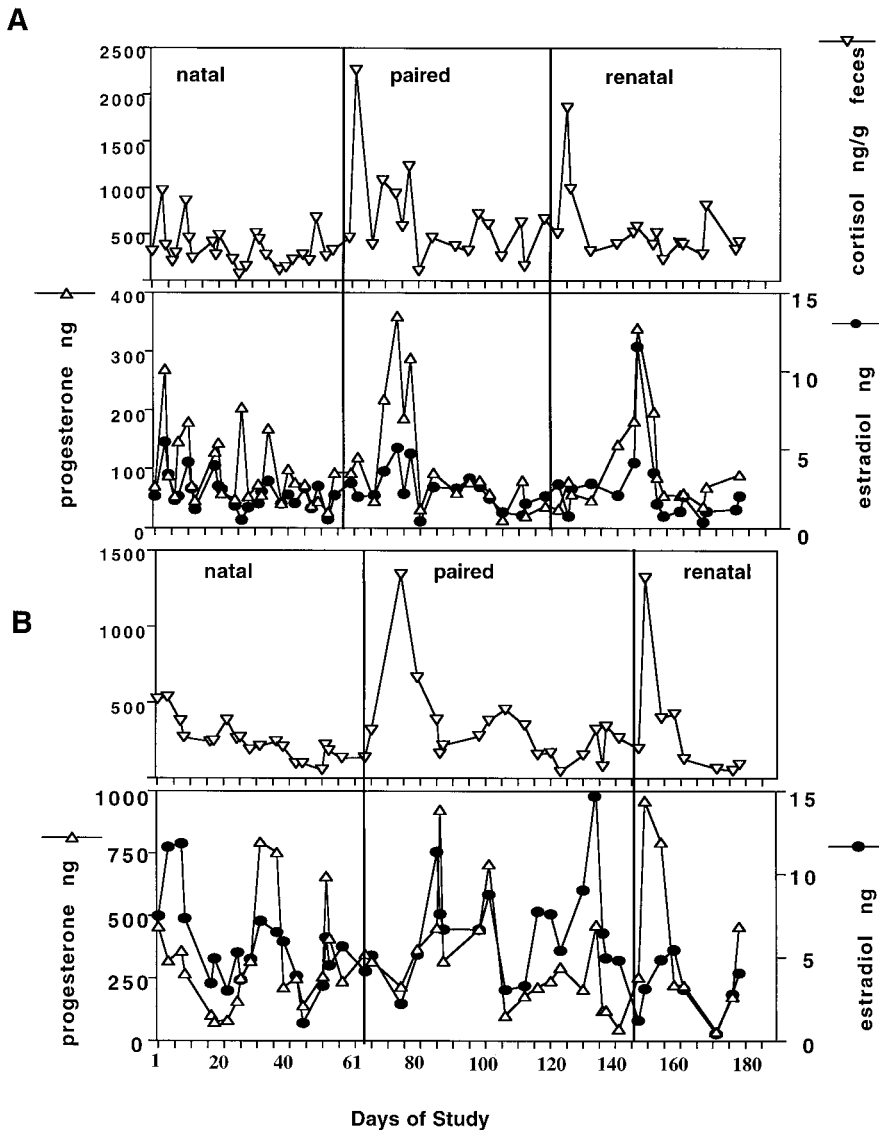


FIG. 4. Fecal cortisol, progesterone, and estradiol concentrations in two daughters. (A) Ovarian cycling began under the paired condition; (B) the female was cycling at the onset of the study.

cycling did not influence the cortisol response to social change. Response to social change has been reported for the cotton-top tamarin (Ziegler *et al.*, 1995; Ginther, Ziegler, and Snowdon, 2001) and for Wied's marmoset (Smith and French, 1997). This chronic secretion of cortisol associated with moves suggests a form of psychosocial stress due to social change. Since some daughters also displayed chronic elevation of cortisol upon returning to their natal cages, it is likely that the females were responding to the move as well as to introduction to a novel male. The elevated cortisol did

not interfere with ovarian cycling since all females but one were cycling during this time.

One of the important findings in this study was the ability of daughters to return to the family group regardless of their reproductive condition. Only one mother showed aggressive behavior toward the returning daughter and this daughter had not shown ovarian cycling. In another family, a younger sister was highly aggressive to the returning female without any involvement from other family members. This suggests that females of postpubertal age may have

opportunities to display reproductive cycling within the natal group and may use the breeding strategy of having extragroup copulations while remaining in the natal group. Araújo (1996) has reported on one female leaving a natural group twice and returning to the group within 45 days. Even though the female had become more peripheral to the group and received aggressive displays, she continued to return to the group. These data also support the findings of Lazaro-Perea (2000) from wild marmoset group encounters when both male and female subordinates were found copulating with other subordinates from other groups. This may be one strategy for remaining in the natal group until an opportunity arises to become a dominant breeding female. Fecal steroids from one group of wild common marmosets indicated that two subordinate females (and most likely daughters) were showing ovarian cyclicity while living with the natal group (Albuquerque *et al.*, 2001). Neither of the subordinate females became pregnant but the dominant female gave birth twice during the study period. Double pregnancies in a group have been reported (Digby and Ferrari, 1994) and may indicate a condition where the subordinate female has participated in extragroup copulations. As suggested by Arruda *et al.* (submitted for publication) in long-term monitoring of wild groups of common marmosets, intergroup encounters may provide subordinate females the opportunity to search for breeding vacancies and establish alliances with extragroup males who may become future breeding partners. This seems likely since females are free to emigrate in the wild (Araújo, 1996).

Daughter marmosets, whether cycling or not, showed no aggressive encounters with either their mother or father. Additionally, the daughters were the ones initiating the affiliative interactions. The lack of aggressive interactions directed at the daughter suggests that there is no obvious behavioral attempt at suppressing reproduction in daughters, unless there is an inhibition of sexual behavior, as suggested by Saltzman *et al.* (1997). However, the daughters in this study showed behavioral changes during ovarian cycling. They were found to scent mark more frequently during the periovulatory period. Interestingly, they also spent more time alone during the periovulatory period.

Through obtaining frequent fecal samples for steroid analyses we were able to monitor endocrine changes in multiple females in the same cage. This allowed us to monitor reproduction without any disruption to the family unit since no captures or venipuncture were required. The fecal steroids clearly defined ovarian cycles and pregnancies. The profiles of

estradiol and progesterone were identical to those described in Ziegler *et al.* (1996), where fecal progesterone showed the first sustained elevation, with estradiol showing a sustained luteal rise several days later. Fecal cortisol also provides important information about reproductive functioning and social transition as seen by its independence of the ovarian steroids following social transition and its tracking of estradiol during ovarian cycling. It is hoped that the information provided in this captive study will provide the framework needed for future endocrine and behavior studies of this species in the wild.

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