

Prolactin Levels during the Periparturitional Period in the Biparental Cotton-Top Tamarin (*Saguinus oedipus*): Interactions with Gender, Androgen Levels, and Parenting

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Unlike biparental bird and rodent species, mating and parenting occur simultaneously in cotton-top tamarins, *Saguinus oedipus*, providing a unique model for examining endocrine interactions. This study was designed to determine the relationship of prolactin to testicular androgens during parenting and mating. Specifically we examined (1) the patterns of postpartum prolactin excretion in male and female tamarins with and without infant survival; (2) the relationship between androgen and prolactin levels during the periparturitional period in male tamarins; and (3) male hormonal response to the postpartum mating that occurs simultaneously with paternal care. All females showed an elevation in prolactin during the first week postpartum and when infants died, female prolactin levels decreased significantly. Infant survival during the first 15 days did not influence male levels of prolactin, cortisol, or the testicular androgens testosterone and dihydrotestosterone (DHT). Prolactin did not show an inverse relationship with any of the androgens (testosterone, DHT) studied during the postpartum period. No significant differences in hormones were found between prepartum levels and the first, second, and third 5-day blocks postpartum for all 10 males. Males who had infant-care experience showed an increase in testosterone during the first 5 days postpartum and prolactin levels correlated with the number of previous births a male had experienced. However, the most significant changes in testosterone occurred in males whose mates ovulated during the first 15 days following birth in contrast to males whose mates ovulated later than 15 days. These results indicate that unlike females, male tamarins are not showing hormonal changes in response to infants. Urinary androgens did not show an inverse relationship to prolactin in male tamarins, but were elevated concurrent with the female's fertile period. © 2000 Academic Press

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Several hormones appear to play an important role in the expression of parenting behavior in males and females but only prolactin has been extensively studied in biparental species. Elevated prolactin levels have been associated with infant care-taking behaviors in many species of mammals and birds. While higher prolactin levels promote maternal care in mammals (Bridges, 1994; Lucas, Ormandy, Binart, Bridges, and Kelly, 1998), prolactin also promotes male parental care behavior in birds (Buntin, 1996), biparental rodents (Brown, 1993), and two species of New World monkeys (common marmoset, *Callithrix jacchus*, Dixon and George, 1982; Mota and Sousa, 2000; cotton-top tamarin, *Saguinus oedipus*, Ziegler, Wegner, and Snowdon, 1996a; Ziegler, 2000). Although prolactin elevations in female mammals at birth appear to be influenced by the prepartum hormonal environment and maintained by the suckling stimulus (Bridges, 1994), male prolactin levels probably respond to different proximate mechanisms.

In many species of seasonally breeding birds, prolactin and testosterone (T) have a reciprocal relationship (Hegner and Wingfield, 1987; Ketterson, Nolan, Wolf, and Ziegenfus, 1992; Logan and Wingfield, 1995). Testosterone levels decline after the breeding season ends, coinciding with the expression of parental care behavior and elevated prolactin levels in males (Schoech, Ketterson, Nolan, Sharp, and Buntin, 1998).

In fact, preventing the decline in testosterone at the onset of parental behavior interferes with the expression of paternal care in dark-eyed juncos (Schoech et al., 1998). It has been proposed that elevated testosterone levels can be detrimental to parenting behaviors if the levels exceed those needed for breeding and are usually associated with heightened aggression as outlined in the "Challenge Hypothesis" (Wingfield, Hegner, Dufty, and Ball, 1990). High levels of testosterone are associated with heightened aggression for maintenance of a breeding territory and mate-guarding behavior during the breeding season (Wingfield and Marler, 1988).

While prolactin and testosterone show an inverse relationship in seasonally breeding biparental birds where parenting and breeding are separated in time, the relationship between these hormones is less clear in biparental rodents. Unlike biparental birds, which breed and care for infants at different times, some rodents ovulate and mate within hours of birth (Reburn and Wynne-Edwards, 1999). In male California mice (*Peromyscus californicus*) living with their mate and pups, plasma prolactin levels were significantly higher than levels in males living with pregnant females or in isolation, but testosterone levels were only marginally lower in males living with their mate and pups than for isolated males (Gubernick and Nelson, 1989). Elevated levels of both testosterone and prolactin have been found in male gerbils (*Meriones unguiculatus*) during their mate's pregnancy, while prolactin levels were elevated and testosterone levels were significantly lower during the postpartum period (Brown, Murdoch, Murphy, and Moger, 1995). In biparental male hamsters (*Phodopus campbelli*), prolactin levels did not increase until several days after the birth of pups, while testosterone levels rose at the end of pregnancy and dropped following birth (Reburn and Wynne-Edwards, 1999). Apparently, not all biparental male rodents show the same prolactin response; in some species prolactin levels are elevated in males before and after parturition (*M. unguiculatus*), while prolactin levels do not increase until after the birth of pups in other species (*Ph. campbelli*, *Pe. californicus*). Testosterone levels appear to decline during the first few days postpartum in all rodent species studied thus far and may be involved in reducing infanticidal aggression (Reburn and Wynne-Edwards, 1999).

Unlike biparental bird and rodent species, mating and parenting occur simultaneously in cotton-top tamarins, providing a unique model for examining endocrine interactions. Father tamarins participate in infant care from the first day of birth through to wean-

ing (Snowdon, 1996) while females ovulate within a range of 13–29 days postpartum with conceptions occurring approximately 84% of the time (Ziegler, Bridson, Snowdon, and Eman, 1987; Ziegler, Widowski, Larson, and Snowdon, 1990). Male tamarins are caring for infants at the time of mating. Evidence from male common marmosets, who are also biparental with postpartum ovulations occurring during parenting, has indicated no reciprocal relationship between prolactin and testosterone during the 10–30 days after infant births (Dixon and George, 1982) while male black tufted-ear marmosets (*Callithrix kuhlii*) show decreased testosterone levels during the peak infant carrying time from the third to fourth week postpartum (Nunes, Fite, and French, in press).

The present study expands on earlier work by assessing the effect of parenting behavior and mating on two other important hormones: the androgen dihydrotestosterone (DHT) and a stress hormone, cortisol. Although DHT is found in lower amounts than testosterone, it is known to be a more potent androgen (Coffey, 1988). DHT has also been linked to changes in sexual behavior associated with androgen production and therefore may be elevated during mating opportunities (Morali, Oropeza, Lemus, and Perez-Palacios, 1994; Mantzoros, Georgiadis, and Trichopoulos, 1995). Cortisol is believed to influence maternal responsiveness to infants (Fleming, Morgan, and Walsh, 1996) and may influence paternal behavior (Taymans, DeVries, DeVries, Nelson, Friedman, Castro, Detera-Wadleigh, Carter, and Chrousos, 1997). We have found that male tamarins who are first-time fathers showed higher levels of cortisol during the first 2 weeks following birth than did experienced fathers (Ziegler, Wegner, and Snowdon, 1996a).

Specifically, we wanted to examine: (1) differences in prolactin secretion between males and females, (2) the relationship of prolactin to testicular androgens during the periparturitional period in male tamarins, and (3) male hormonal response to mating during the parenting period. We predicted that, similar to other mammals, female tamarins would show elevated prolactin levels at the end of pregnancy (Hodson, 1996). Additionally, females with suckling infants were expected to have significantly higher prolactin levels than females whose infants died. Based on a previous study of prolactin levels in male cotton-top tamarins (Ziegler et al., 1996a), we did not expect the amount of carrying time to influence prolactin levels. Since cotton-top tamarin males show immediate parenting behaviors and elevated prolactin levels prior to an infant's birth, we do not expect to see a major increase in

prolactin levels immediately after birth and therefore, we would not expect to see significant changes in testosterone.

METHODS

Subjects

Pre- and postpartum urinary levels of prolactin were examined in 12 adult female cotton-top tamarins: 6 females who had lost infants within the first week of birth and 6 females who were suckling infants. Urine samples were collected on these females during the week prior to birth and during the following 6 weeks postpartum. Samples were collected from 10 adult breeding males during the 15 days prior to and the 15 days following the birth of infants. Samples were also collected from their mates (some were the same females in the above study) during the postpartum period to determine when the postpartum ovulation occurred. The tamarins were housed in the Tamarin Colony of the University of Wisconsin Psychology Department. All tamarins lived either in large family cages ($3.0 \times 1.8 \times 2.3$ m) where they were the dominant breeding male or female or in pair cages ($1.5 \times 0.85 \times 2.3$ m) with their mate and any offspring. The tamarins were maintained on a 12-h day/night cycle, typical for their equatorial origins. Diet and husbandry techniques for the colony have recently been described (Ginther, Ziegler, and Snowdon, in press). All males and females in the study were between 3 and 14 years of age and all but two pairs had experience with previous births. Three to four urine samples were collected from 3 additional cotton-top tamarin males with no infant experience housed at the Wisconsin Regional Primate Research Center (WRPRC) for prolactin measurement. These males were between the ages of 5 and 6 years. The WRPRC-housed tamarins were caged either individually (1 male) or as a male-male pair (2 males) in cages measuring $61 \times 91 \times 183$ cm. The tamarins were on a 12-h light/dark cycle and were fed once per day as described in Saltzman, Severin, Schultz-Darken, and Abbott (1997) for marmosets.

Behavioral and Hormonal Sample Collection

To determine the amount of time mothers and fathers carried infants during the first 15 days following birth, instantaneous scan samples on the identity of the tamarin carrying the infant (or infants) were re-

corded five times per day: at wake-up $\sim 8:00$, $10:00$, $12:00$, $14:00$, and $\sim 16:00$. Immediate observation of infants and their caretakers was made directly upon entering the room where the family lived. This allowed for observation of the infants' position before reaction to the observer's presence could affect their behavior. Carrying data were expressed as a percentage of all observations possible during each block of 5 days (days 1-5, 6-10, and 11-15 postpartum), which provided for a percentage of all opportunities to carry within the time period. If there were two infants and one was carried by each parent, then both parents were scored. Since we are interested in the relationship between hormonal levels and direct parental contact with infants, we did not include infant contact with other family members in analyses although it was recorded. In a previous study, instantaneous scan sampling of percentage of time carrying was found to be as reliable as focal animal sampling ($r = 0.89$, $n = 20$, $P < 0.001$; Ziegler *et al.*, 1990).

Urine samples were collected noninvasively from the tamarins as first morning void. The lights were turned on in the rooms where the tamarins lived between 8:00 and 9:00 AM and urine was collected by holding a bucket underneath the tamarin of interest (Ziegler *et al.*, 1987). All collected urine was mixed in the collection bucket prior to centrifugation and storage at -20°C . For the urine aliquot selected for protein hormone analysis, glycerol at 0.52 M was added to the sample prior to freezing (Ziegler *et al.*, 1996a). Samples remained frozen until hormonal analyses. Prior to the birth of infants, urine samples were collected three times per week. Following birth, urine was collected daily for females and three times per week for males.

Hormonal Analyses

To examine the role of nursing on prolactin levels, all female urine samples were analyzed for urinary prolactin. Urine was also collected postpartum from the mates of the male tamarins in this study for luteinizing hormone (LH) determination of the postpartum ovulation. All male urine samples were analyzed for prolactin, testosterone and DHT to determine their relationships to one another and to male infant carrying. Cortisol was measured in the males to assess whether births were associated with increased stress levels (Ziegler, Scheffler, and Snowdon, 1995; Ziegler *et al.*, 1996a). All urine samples were analyzed for creatinine levels prior to hormonal analyses by the methods reported in Ziegler *et al.* (1995). Hormone values were divided by creatinine levels to correct for

fluid variability between samples and expressed as milligrams of creatinine (mg Cr).

Urinary prolactin analysis has been described in Ziegler *et al.* (1996a). The following modifications were made to the assay for this study. The volume of urine samples was increased to 1.5 ml. The standard human prolactin is now supplied by NHPP (National Hormone and Pituitary Program) and standards are measured from 0.1 to 10 ng. The concentration of I^{125} trace (DuPont) was changed to 100 μ l of 10,000 cpm and the antibody concentration was changed to a pipetting concentration of 1:2500 yielding approximately 30% binding. The antibody is made up in 2.5% normal rabbit serum. The second antibody is now an "In House" preparation of goat anti-mouse gamma globulin (AR2202, Biogenesis, Brentwood, NH) and used as a 3% solution in assay buffer and pipetted as 500 μ l/tube. The second antibody is applied on the second day and allowed to sit overnight. The tamarin urine pool was revalidated following the extensive changes in the assay. Accuracy was found to be $106.6 \pm 2.5\%$ SEM, $n = 6$ points in triplicate and serially diluted tamarin urine was parallel to the human prolactin standard, $t = -1.67$, $df = 26$, $P > 0.05$. All samples for an individual were run in a single assay. Mean intra-assay coefficients of variation (CVs) for two tamarin pools were 7.1 and 10.9%. Mean inter-assay coefficients of variation were 25.4 and 12.1%, respectively, $n = 11$ and $n = 6$.

Cortisol concentrations were determined for males and females by direct enzyme immunoassay (EIA) on the urine samples according to the methods described and validated in Ziegler *et al.* (1995). Mean intra-assay CVs for a high and low pool were 2.6 and 2.3%. Mean inter-assay CVs were 6.0 and 10.3%, $n = 16$.

LH measurement for the determination of the timing of the postpartum ovulation was carried out according to the method reported in Ziegler, Matteri, and Wegner (1993) with the replacement of the rhesus reference standard with purified human CG (chorionic gonadotropin), CR-127, NHPP. Validation of tamarin urine with the new standards gave an accuracy of $103 \pm 2.4\%$, SEM, $n = 5$ points in triplicate, and parallelism was determined by showing no difference in slopes between serial dilutions of tamarin urine and concentrations of human CG standards at 0.1–5 ng, $t = 0.84$, $df = 23$, $P > 0.05$. This method is routinely run in our laboratory and standard CVs are 3.6% for intra-assay variation and 6.5% for inter-assay variation.

Urinary testosterone and DHT reflect testosterone secretion since both steroids have been shown to in-

crease in tamarin urine following administration of exogenous testosterone (Ziegler, Carlson, Ginther, and Snowdon, 2000). The measurement of testosterone and DHT required prior separation by chromatography since antibodies to testosterone cross-react 100% with DHT. Additionally, direct measurement of testosterone did not correlate highly with column separated measurement ($r^2 = 0.71$), indicating that the antibody cross-reacts with something other than testosterone and DHT in tamarin urine. Column chromatography separation for T and DHT was performed by the methods described and validated by Ginther *et al.* (in press). Briefly, since 94% of urinary testosterone in male tamarins has been shown to be excreted as double conjugates, the samples underwent solvolysis prior to ethyl acetate extraction (Ziegler *et al.*, 2000). Aliquots of 500 μ l were subjected to solvolysis according to the method described in Ziegler, Scheffler, Wittwer, Schultz-Darken, Snowdon, and Abbott (1996b). Samples were then reconstituted in 1 ml of 4% ethyl acetate/isooctane. Testosterone and DHT were separated by celite column chromatography using the System I technique as described in Abraham, Buster, Lucas, Corrales, and Teller (1972) with the following modifications. Samples were applied to the column after sonification in an ultrasound cleaner. The application tubes were rinsed with 0.5 ml of 4% ethyl acetate/isooctane and applied to the column. A rinse of 3.5 ml isooctane was run through the column and discarded. The DHT was eluted with 4.0–4.5 ml of 10% ethyl acetate/isooctane, and the testosterone was eluted with 4.0 ml of 20% ethyl acetate/isooctane. The separated samples were dried under air in a water bath and resuspended in 500 μ l ETOH. Recoveries for the sample preparation (including solvolysis, extraction and chromatography) were $92 \pm 1.5\%$, SEM, $n = 7$, for tritiated testosterone and $81.4 \pm 3.3\%$, SEM, $n = 8$, for tritiated DHT.

The EIAs for testosterone and DHT are modifications of the EIA technique described by Munro and Stabenfelt (1984). The testosterone antibody (AB156) cross-reacts over 90% with DHT. Therefore, the same antibody and testosterone-tagged enzyme were used for each assay with different standards. Methods for tamarin urine have been described by Ginther *et al.* (in press). Serially diluted pooled urine samples were parallel to the standards for testosterone ($t = -0.74$, $df = 60$, $P > 0.05$) and to the standards for DHT ($t = -1.495$, $df = 28$, $P > 0.05$). Accuracy for testosterone was measured at $102.6 \pm 2.4\%$, $n = 8$, and for DHT was measured at $98.7 \pm 0.4\%$, $n = 9$. Mean intra- and inter-assay CVs for testosterone were 2.3 and 16.1%,

respectively, and for DHT they were 1.8 and 15.2%, respectively.

Statistical Analysis

Prolactin excretion in females. To test the role of nursing on prolactin levels, the concentration of prolactin per mg Cr for each sample was averaged per week for each female. All samples were numbered relative to the day of birth. The day of birth was day 1 for the first week postpartum and the 7 days before the day of birth were considered to be the last week of pregnancy. Nonparametric tests were used for the analysis. The Wilcoxon Signed Ranks Test was used to compare pre- and postpartum prolactin levels for all females. The Mann-Whitney *U* Test was used to compare mean prolactin levels between females who kept their infants and females who lost their infants for the entire postpartum period and by weeks. Friedman's ANOVA was used to test females across weeks for those that nursed and across weeks for those that lost infants. The percentage change in prolactin levels from week 1 to other weeks was analyzed by the Wilcoxon Signed Ranks Test.

Hormonal levels in males. For analysis of male hormones, the day of birth was considered to be the first day postpartum. The number of days on either side was counted from this day to the 15th day from birth. This allowed us to examine the hormones in the urine samples by averaging the hormone concentrations of each sample during each 5-day block: three blocks prior to the day of birth and three blocks after birth. Averages were reported as means \pm SEM. Hormonal data from the 30-day collection of male urine were divided into prepartum levels and three 5-day postpartum blocks for analyses. We divided the postpartum data into 5-day blocks to examine the relative change of hormones from birth through the timing of the postpartum ovulation. The percentage change was calculated for each hormone from its prepartum levels (i.e., prepartum levels were set at 100%). Significant changes from prepartum levels were determined by the Wilcoxon Signed Ranks Test. Mann-Whitney *U* analyses were used for comparisons of hormone levels between males who kept or lost their infants, for hormonal changes between males whose females ovulated early versus males whose females ovulated late, and for prolactin levels between males with no experience and those with one or two births. In each sample within a male as well as on averaged data across all males, the Pearson product moment coefficient of correlation was used to determine the relationship

between prolactin and the testicular androgens. Correlation was also used between average hormone change per 5-day block and the percentage carry time. Friedman's two-way ANOVA was used to test hormone levels across time conditions. The Wilcoxon Signed Ranks Test was used to compare prepartum hormone levels with each time block for each hormone. *P* values <0.05 were considered significant.

RESULTS

Hormonal Changes with Infant Survival Patterns

Mean prolactin levels in all females, regardless of whether the infants survived, increased significantly from the week prior to birth to the first week postpartum [$Z = 2.85$ (12), $P = 0.004$]. Mean prolactin levels averaged over the entire postpartum period for each female were significantly higher for the nursing mothers than for mothers who lost infants [$U = 33$ (6,6), $P = 0.016$]. For females who lost their infants, prolactin levels were significantly lower than in nursing females during the second [$U = 32$ (6,6), $P = 0.025$], third [$U = 34$ (6,6), $P = 0.01$], fourth [$U = 35$ (6,6), $P = 0.006$], and fifth weeks [$U = 26$ (6,5), $P = 0.04$] but not during the sixth week (Fig. 1A). The percentage change in weekly mean prolactin levels for female tamarins before and after birth based on infant survival is found in Fig. 1B. The percentage change in prolactin levels from week 1 was near significance during week 3 [$Z = 1.78$ (6), $P = 0.075$] and significantly different during week 4 [$Z = 2.2$ (6), $P = 0.028$]. Timing of the postpartum LH peak for nursing females and females who lost infants indicated that nursing females took longer to ovulate (23.7 ± 4.73 days) than nonnursing females (15.2 ± 2.26 days) and this neared significance [$U = 25$ (6,5), $P = 0.065$].

In contrast to females, mean prolactin levels did not change in males from the week before birth to the week following (before: 0.98 ± 0.22 ng/mg Cr; after: 1.08 ± 0.20 ng/mg Cr). There was no difference in mean prolactin levels for the entire 15 days before (1.03 ± 0.24 ng/mg Cr) and after (1.06 ± 0.20 ng/mg Cr) the birth of infants. Offspring survival also did not influence the levels of prolactin in the males during the 2 weeks following birth (Fig. 2). Prolactin levels for fathers whose infants survived were no different than prolactin levels for fathers whose infants did not. Other hormones measured also showed no difference in concentration pre- and postpartum for the 10 males:

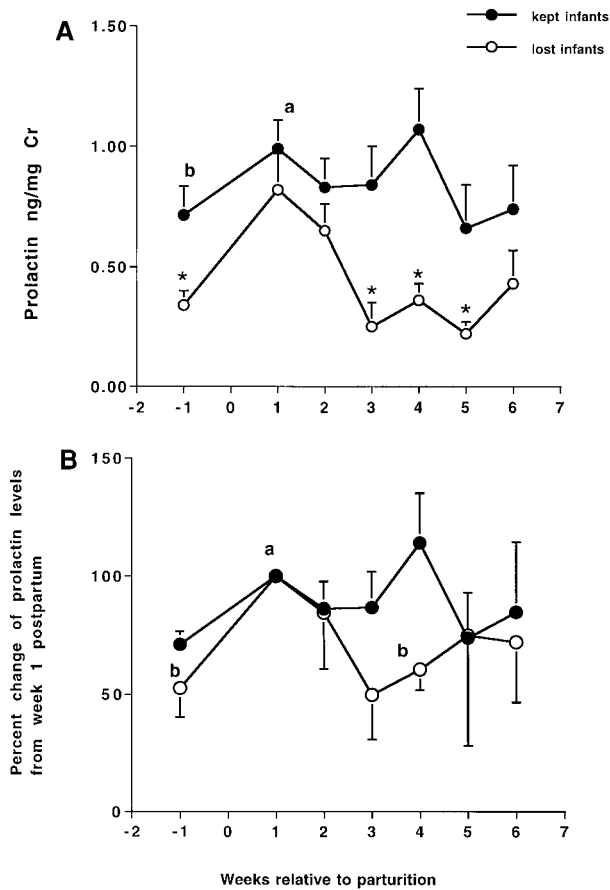


FIG. 1. Weekly prolactin levels in cotton-top tamarin females who lost infants after birth or kept infants and nursed. (A) Mean \pm SEM change in prolactin levels from week 1 postpartum averaged by week before and for the 6 weeks following parturition. All females had significantly higher levels of prolactin on the week after birth (a) compared to the week before (b) ($Z = 2.85$, $P = 0.004$). Females who lost their infants had significantly lower levels of prolactin during weeks -1, 2, 3, and 4 (significance indicated by *) than females who kept infants. (B) Mean \pm SEM percentage change in prolactin levels from the first week postpartum for females who lost or kept infants. For females who lost their infants, week 1 (a) was significantly different from week -1 and week 4 (b) and near significance for week 3.

prepartum cortisol $9.38 \pm 1.24 \mu\text{g}/\text{mg Cr}$, postpartum cortisol $10.00 \pm 1.14 \mu\text{g}/\text{mg Cr}$; prepartum testosterone $9.01 \pm 3.53 \text{ ng}/\text{mg Cr}$, postpartum testosterone $9.53 \pm 3.42 \text{ ng}/\text{mg Cr}$; prepartum DHT $0.22 \pm 0.07 \text{ ng}/\text{mg Cr}$, postpartum DHT $0.28 \pm 0.05 \text{ ng}/\text{mg Cr}$.

Table 1 provides age, experience, offspring survival data, and percentage carrying time for the 10 fathers in this study. Fathers carried infants as much as their mates did in the first 5 days (no difference in percentage carrying time between paired males and females).

Males with one versus two infants did not differ in percentage time carrying for the first 5 days, for days 6–10, or for days 11–15.

Testosterone levels did not differ between fathers raising infants and fathers without infants to carry during the week before infants were born or during days 1–5, days 6–10, or days 11–15 (Fig. 2). Mean testosterone levels for the entire postpartum period did not differ between fathers who kept both infants ($10.32 \pm 5.9 \text{ ng}/\text{mg Cr}$) and fathers who lost one infant ($12.05 \pm 9.4 \text{ ng}/\text{mg Cr}$). Additionally, changes in testosterone did not correlate with the amount of time fathers spent carrying infants. No significant correlation was found for testosterone and percentage carrying time, for DHT and percentage carrying time, or for prolactin levels and percentage carrying time.

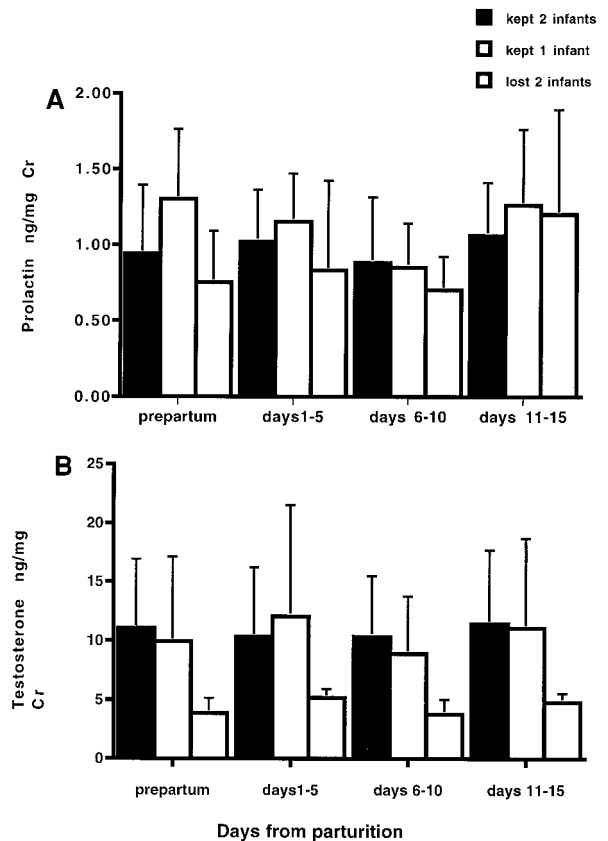


FIG. 2. Mean \pm SEM levels of urinary (A) prolactin and (B) testosterone in 10 male cotton-top tamarins classified as to whether they kept two infants, kept one of two infants, or lost both infants. The 15 days prior to birth were averaged for each male and are presented as the mean prepartum levels. No significant difference in prolactin levels or testosterone occurred whether the males kept or lost infants.

TABLE 1

Data on Cotton-Top Tamarin Males for Age (Years), Experience, Carrying Effort, and Time to Female Ovulation

Infants	Males	Age	No. of previous births	Female ovulation ^a	% carry		
					1-5 days	6-10 days	11-15 days
Kept 2	AR	7.5	8	14	30	20	25
	JU	12.0	10	14	41	33	30
	YA	3.0	0	37	65	76	79
	NK	4.5	2	15	52	21	29
			Mean	20	47 ± 7.5	37.5 ± 13.2	40.8 ± 12.8
Kept 1	DI	3.8	2	24	79	22	42
	RH	13.5	15	15	30	5	25
	TI	7.5	4	20	67	13	0
	DU	3.0	1	39	24	0	36
			Mean	24.5	50.0 ± 13.6	10.0 ± 4.8	25.8 ± 12.8
Lost 2	YE	4.0	2	13	0	0	0
	NI	7.0	3	17	9.5	0	0
			Mean	15	4.8 ± 4.8	0	0

^a The interval in days from parturition to the day of the LH peak in the female tamarin.

Relationship of Prolactin to Testicular Hormones

Prolactin's relationship to testosterone and DHT was tested both within males and between males. No significant inverse relationship between testosterone and prolactin concentration was found in males. The relationship of prolactin to testosterone or of prolactin to DHT for all samples was tested for the 10 individual males. Prolactin levels from only 3 males showed a negative correlation with testosterone and these correlations were not significant ($P > 0.20$). A significant negative correlation with DHT was found for prolactin levels from only 1 male (RH, $r = -0.60$, $P = 0.03$). For the other males, both positive and negative relationships occurred but none of them were significant. A comparison of the mean levels of prolactin for all males with mean testosterone and mean DHT levels showed no clear inverse relationship. For individual males, the ratio of testosterone to prolactin or of DHT to prolactin showed no significant differences across the blocks of days.

The range of concentrations for testosterone and DHT varied between males. Seven of the 10 males showed testosterone levels in a similar range (0.5 to 9 ng/mg Cr) while 3 of the males had testosterone levels 10 times higher (12 to 50 ng/mg Cr). For those males with higher testosterone levels, DHT levels were higher as well. Due to such variation between males, the remaining analyses were performed using percentage change from the prepartum levels (100%).

None of the hormones measured showed a significant increase across postpartum blocks (days 1-5,

6-10, and 11-15) from prepartum levels (Fig. 3). Friedman's two-way ANOVA indicated that prolactin did not vary across blocks, cortisol did not vary across blocks, and testosterone did not vary across blocks nor did DHT.

Experiential and Mating Effects on Hormonal Levels

Table 1 indicates the parenting experience of each male. Males raising their first set of infants were considered inexperienced while males who had raised multiple sets were considered experienced. Eight of the 10 males were defined as experienced males and 2 males were inexperienced. Experienced males showed no significant change across blocks in any of the hormones measured. However, Wilcoxon paired comparisons indicated that testosterone was elevated significantly from prepartum levels on days 1-5 for experienced males [$Z = 2.4$ (8), $P = 0.02$].

Experience was related to prolactin levels. The concentration of prepartum prolactin was highly correlated to the number of previous births a male had experienced ($y = 5.53x - 1.04$; $r = 0.88$, $P = 0.0001$). Age of the male also correlated with prepartum prolactin levels ($y = 3.80x + 3.16$; $r = 0.87$, $P = 0.0001$). However, when comparing males an average of 5 years old with no previous birth experience (including the Primate Center tamarins) to younger males with one to two previous births, prolactin levels were significantly lower in the inexperienced males

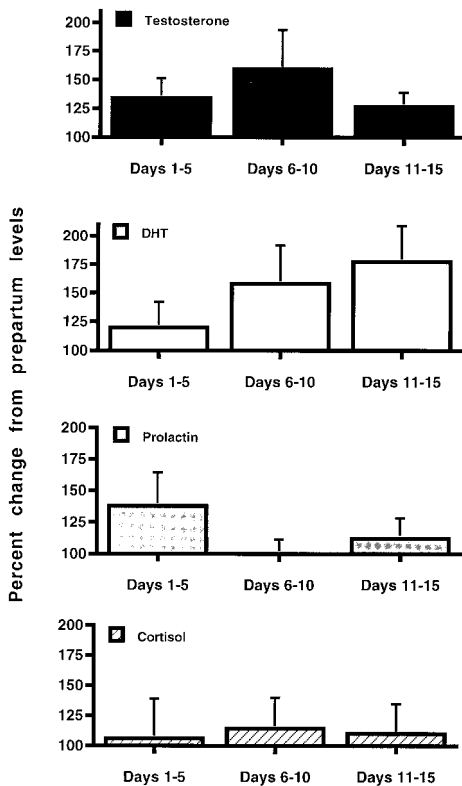


FIG. 3. Mean \pm SEM percentage change in urinary testosterone, DHT, prolactin, and cortisol from prepartum levels for 10 male cotton-top tamarins. The postpartum days were divided into days 1–5, days 6–10, and days 11–15 following parturition. No significant change from prepartum levels occurred for any hormone.

than in males who were younger [Fig. 4; $U = 0, (4, 4), P = 0.02$].

Data from male tamarins were separated into data for those males whose females ovulated within the first 15 days when hormonal samples were collected from males versus data for those males whose females ovulated after the end of collection (Table 1). Females that ovulated within 15 days of parturition were classified as early ovulators while those who had postpartum ovulation later than 15 days were classified as late ovulators. Mates for five of the males had a urinary LH peak indicative of early ovulation (14.2 ± 0.37 days), while five mates did not show LH peaks until more than 15 days after birth (27.4 ± 4.47 days) and were classified as late ovulators. The age of males with early ovulators was not significantly different from that of males with late ovulators. Significant differences were found for testosterone across the time blocks in males paired with early ovulators [$Fr = 10.68 (5), P = 0.01$; Fig. 5]. The percentage change in testosterone for the males with early ovulators was

significantly higher than prepartum levels during days 1–5 ($Z = 2.02, P = 0.04$), days 6–10 ($Z = 2.02, P = 0.04$), and days 11–15 ($Z = 2.02, P = 0.04$). Comparisons of early and late ovulators indicated that testosterone changes from postpartum levels were significantly higher in the early ovulators during days 1–5 [$U = 23.00(5, 5), P = 0.03$], and days 6–10 [$U = 24(5, 5), P = 0.02$] and approached significance on days 11–15 [$U = 21(5, 5), P = 0.08$]. Males paired with early ovulators showed no significant change in DHT across the three blocks and DHT percentage change was not significantly higher in the males with early ovulators than in the males with late ovulators during days 1–5, days 6–10, and days 11–15. Neither prolactin nor cortisol showed significant changes across the postpartum time blocks.

DISCUSSION

Our study indicates that hormonal levels in male tamarins do not significantly change in response to the birth and infant stimuli while female tamarins show significant changes in response to the birth. It is possible that in male tamarins hormonal changes occur prior to infant births and, therefore, influence parental behaviors in males who are carrying immediately after birth. Tamarin males show elevated levels of prolactin before and after infant birth (Ziegler et al., 1996a) with an increase in androgens prior to their mate's ovulatory period. Parenting behavior and postpartum mat-

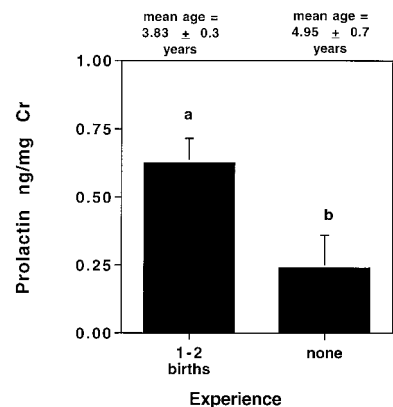


FIG. 4. Comparison of mean \pm SEM prolactin levels for males who had one or two previous birth experiences with an average age of 3.83 years to males with no previous birth experiences with an average age of 4.95 years. Males with experience had significantly higher levels of prolactin ($U = 0.0, P = 0.02$) than inexperienced males while age remained constant.

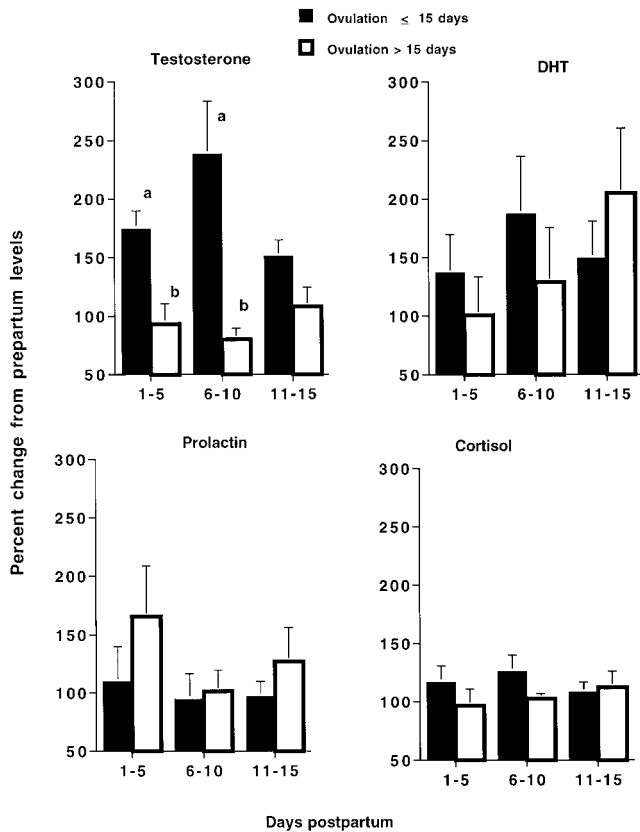


FIG. 5. Mean \pm SEM percentage change from prepartum hormone levels to postpartum levels during days 1–5, 6–10, 11–15 for five males whose females ovulated early (≤ 15 days postpartum) versus five males whose females ovulated late (> 15 days postpartum). Testosterone levels were significantly higher in the males with early ovulators than in males living with late ovulators at days 1–5 [$U = 23(5,5)$, $P = 0.03$] and days 6–10 [$U = 24(5,5)$, $P = 0.02$] and were nearly significant on days 11–15 [$U = 21(5,5)$, $P = 0.08$].

ing occur simultaneously in the cotton-top tamarin. Male tamarins actually mount and copulate with their mates during the postpartum ovulation while carrying infants on their back (personal observation). In our colony, breeding males do not have a reciprocal relationship of prolactin to testosterone during early parenting and testosterone levels associated with breeding do not interfere with paternal behavior.

Influence of Infant Survival on Hormones

The contrast in male and female prolactin responses to infant survival suggests that different mechanisms control male and female prolactin secretion during parental care in cotton-top tamarins. As has been re-

ported for other species where prolactin secretion is facilitated by gestational changes in estrogens and progestins (Bridges, 1996), prolactin levels in female cotton-top tamarins increased at the end of pregnancy. Postpartum prolactin levels remained elevated only for those females with surviving infants, consistent with previous work showing that the suckling stimulus causes marked increases in the secretion of prolactin (Tucker, 1988). In contrast, we found little difference in prolactin levels between males who carried infants and those who did not (because their offspring had died) for the first 2 weeks. Our findings suggest that male prolactin secretion was not dependent on infant survival.

Male cotton-top tamarins carried infants as much as females did during the first 5 days postpartum. Even males with four to five subadult offspring present in the family cage still carried 30–50% of the time during the first 5 days. If elevated prolactin levels facilitate parental behaviors in males, then prolactin levels should have increased prior to parturition in cotton-top tamarins since males carry on the first day of birth. This may be why we did not see differences in prolactin levels before and after birth. Not all callitrichid species show such extensive paternal involvement with infants during the first week. Common marmoset, pygmy marmoset (*Cebuella pygmaea*), black tufted-ear marmoset (*C. kuhlii*), and buffy-headed marmoset (*Callithrix flaviceps*) mothers do most of the infant carrying during the first week (Wamboldt, Gelhard, and Insel, 1988; Koenig and Rothe, 1991; Ferrari, 1992; Rothe, Darms, Koenig, Radespiel, and Juenemann, 1993; Nunes *et al.*, in press) as do mothers in *Leontopithecus* species (Hoage, 1982; Souza de Oliveira, Lopes, Alonso, and Yamamoto, 1999). Differences in male infant care between species may affect patterns of prolactin secretion. Unfortunately, no prolactin data exist for the above callitrichid species, other than the common marmoset. Recent data from male common marmosets indicate that circulating prolactin levels in fathers were higher in the 8 weeks following parturition than during the 8 weeks prepartum (Mota, Sousa, and Box, 1999). Male marmosets also showed higher levels of prolactin with increased infant contact time (see also Dixson and George, 1982). This contrasts with male tamarins where prolactin levels were elevated prior to infant caretaking and measurement of accumulated daily levels did not show a response to contact time.

Experiential and Mating Effects on Hormones

Prolactin levels were correlated to the number of previous births a male had experienced, suggesting, therefore, that experience with one's own infants does play a role in prolactin elevation in cotton-top tamarins. Prolactin levels of experienced males appear to respond more to prepartum stimuli while levels in inexperienced males respond to postpartum stimuli (Ziegler et al., 1996a). Although little data are available in mammals, parenting experience has been shown to influence prolactin's facilitation of parenting in birds. Prolactin-treated experienced ring doves exhibited a much higher frequency of parental behaviors than inexperienced ring doves (Wang and Buntin, 1999). In general, experience may interact with prolactin to enhance its effects on parenting behaviors in both sexes. In our colony, age has been a confound in examining the number of previous births with prolactin levels since males are getting older with each birth of infants. However, the data in Fig. 4 show that when older males with no previous exposure to infants are included, prolactin levels remain low. This clearly indicates that prolactin levels are not changing with age but are changing with a tamarin male's parental experience.

Patterns of hormonal secretion and mating in cotton-top tamarins appear to diverge from the patterns in biparental rodents and birds. While all three groups show increased levels of androgens during the mating period, the timing of copulation with respect to parental care periods differs. Male Mongolian gerbils and dwarf hamsters have elevated testosterone levels at the end of pregnancy before mating starts in order to facilitate mating as early as the day of birth (Brown et al., 1995; Reburn and Wynne-Edwards, 1999). Therefore, testosterone changes are consistent with a mating response. Unlike birds and rodents, cotton-top tamarins mate throughout the parental care phase. Postpartum ovulations in female tamarins generally occur 12–20 days after birth, and conception rates are high (over 84%, Ziegler et al., 1987). For this reason, testosterone levels are most likely at reproductively functional levels during the early parenting phase. Increased levels of androgens occurring during the first 15 days postpartum may reflect an association with mating patterns.

Few changes were found for cortisol and DHT from pre- to postpartum levels. DHT levels showed a tendency to increase in males living with early ovulators but this was not significant. It may be that DHT ele-

vations are responsive to the actual copulatory behaviors and, therefore, would be seen as acute changes. The testosterone elevations were chronic, lasting more than 5 days. Since we did not collect daily urine samples, we cannot determine DHT responses to the actual timing of the females' postpartum ovulation or for days associated with copulatory behaviors. No clear changes in cortisol levels were found between pre- and postpartum conditions for males regardless of classification. However, cortisol levels were higher in inexperienced males than in the experienced males, consistent with our previous study (Ziegler et al., 1996a). Cortisol levels did not change in response to other hormones but were responsive to changes in social conditions, such as taking care of one's own infants for the first time. Infant carrying time was not elevated with the elevation of cortisol levels in inexperienced fathers and, therefore, may not facilitate responsiveness to infants in males as has been reported for maternal responsiveness (Fleming et al., 1996).

In summary, males show very different hormonal profiles from females during the periparturitional period. Since there are no significant changes in hormone levels from pre- to postpartum, males may be responding to hormonal changes that occur earlier in the female's pregnancy. Male cotton-top tamarins are prepared to begin paternal care immediately following the birth of infants. However, this does not influence their ability to mate and produce significant changes in testosterone levels with mating behavior. Simultaneous mating and parenting provide cotton-top tamarins with the ability to obtain a high reproductive potential and yet continue to have a high investment in infant development.

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