

Responsiveness of expectant male cotton-top tamarins, *Saguinus oedipus*, to mate's pregnancy

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Abstract

In the cotton-top tamarin, a primate where paternal care is critical to the survival of the offspring, we found that expectant fathers experienced multiple hormonal changes during their mate's pregnancy. Fathers that had experienced several previous births showed significant changes in urinary estrogens, androgens, prolactin and cortisol in the last 2 months before birth, whereas less-experienced fathers (LEF) did not. The female's midpregnancy rise in glucocorticoids was followed within 1–2 weeks by a peak of cortisol and corticosterone in her paired male in 70% of all males and 100% of all experienced males. Examination of behavioral interactions between the pairs did not reveal changes in rates of interactions between the experienced pairs over pregnancy. However, the less-experienced pairs had significantly higher levels of affiliative and sexual interactions. Therefore, behavioral communication between the pair did not appear to account for the hormonal changes occurring within the experienced fathers (EF). The midpregnancy rise of glucocorticoids in females may stimulate a glucocorticoid response in male tamarins and thereby activate other hormonal changes in males to prepare them for their parenting role.

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Introduction

Maternal care is critical to the survival of a mammalian offspring. However, in many socially monogamous mammalian species, infant care duties, except lactation, are shared between both mother and father (or in cooperative breeding species, the entire group). Monogamous males demonstrate a variety of parenting behaviors that promote infant survival or may lead to improved growth (Gubernick and Teferi, 2000; Wynne-Edwards, 1998). Paternal behaviors include assistance in infant birth, licking, retrieval and thermoregulation of Djungarian hamster (*Phodopus campbelli*, Jones and Wynne-Edwards, 2000), Mongolian gerbil (*Meriones unguiculatus*, Brown et al., 1995) and California mouse pups (*Peromyscus californicus*, Gubernick and Nelson, 1989), carrying, protecting and food sharing with New World primate infants, that is, marmosets and tamarins (*Callithrix jacchus*, *Callithrix kuhli*, *Saguinus oedipus*,

Nunes et al., 2001; Pryce, 1995; Snowdon, 1996; Tardif et al., 1990). Both experience and hormonal changes appear to be involved in promoting paternal care behaviors.

In females, an increase in experience leads to increased infant responsiveness (Bridges, 1996; Fleming et al., 1997). In both sexes, tamarins' experience in caring for younger siblings before becoming a parent increases survivorship of one's own infants (Epple, 1978; Johnson et al., 1991; Tardif et al., 1984). Survival rates for common marmosets and cotton-top tamarins are consistently higher as a function of both prior experience and parity (for review, see Snowdon, 1996). However, in marmoset and tamarins, raising one's own infants appears to be a different type of experience. Multiple experiences with infants may allow monogamous males to learn to tolerate infants as well as to perceive cues of infant's needs (Fleming et al., 2002). Therefore, with experience, males learn parenting skills.

Previous work from our lab has shown that experience is related to prolactin levels. The more births a father has experienced, the higher his prolactin levels (Ziegler et al., 1996a). This is independent of the male's age (Ziegler et al., 2000b). In human fathers, experienced fathers had higher prolactin levels than first-time fathers when hearing infant

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cries and both experience and testosterone levels contributed to a father's response to infant cries (Fleming et al., 2002). These studies on humans and tamarins indicate an interactive component between experience and hormonal changes.

Hormones known to influence maternal behavior in mammals have been examined in a growing number of socially monogamous males (for review, see Fleming et al., 2002; Ziegler, 2000). Hormonal changes in parental males have been documented not only during early infant care but also in expecting fathers before his offspring's birth. Prolactin levels are elevated during infant caregiving in male Mongolian gerbils (Brown et al., 1995), California mice, (Gubernick and Nelson, 1989), Djungarian hamsters (Reburn and Wynne-Edwards, 1999), common marmosets (Dixson and George, 1982; Mota and Sousa, 2000) and cotton-top tamarins (Ziegler et al., 1996a). Are these hormonal changes due to infant contact? Common marmoset fathers show higher prolactin while carrying infants (Dixson and George, 1982; Mota and Sousa, 2000) and experienced human fathers show higher prolactin levels in response to infant cries (Fleming et al., 2002). Conversely, Goeldi's monkey, *Callimico goeldii*, shows elevated urinary prolactin levels postnatally but before fathers begin to carry infants indicating that in this species, prolactin elevation may not be associated with infant contact (Schradin and Anzenberger, 2002; Schradin et al., 2003). Fathers or other helpers do not carry infants until the third week postpartum in Goeldi's monkeys. In several species, testosterone levels are lower postpartum than prepartum; male Mongolian gerbils (Brown et al., 1995), Djungarian hamsters (Reburn and Wynne-Edwards, 1999), human fathers (Berg and Wynne-Edwards, 2001, 2002; Storey et al., 2000) and black tufted-ear marmosets (Nunes et al., 2001), but not for the common marmoset where no change in testosterone has been associated with infant carrying (Dixson and George, 1982) or in the cotton-top tamarin where testosterone levels 2 weeks pre- and postpartum did not differ (Ziegler et al., 2000b).

Prenatal hormonal changes have been found in some expectant fathers. Fathers of biparental species may be responsive to cues from the pregnant female and hormonal changes may lead to increased paternal motivation. However, this has not been tested (Ziegler and Snowdon, 2000). Experienced cotton-top tamarin expectant fathers showed significant elevations of prolactin in the third month of a 6-month gestation and significant elevations of testosterone in the fourth and fifth month before birth. In expectant human fathers, cortisol levels are higher during late gestation and testosterone levels are elevated in late pregnancy when compared to postnatal hormone levels (Berg and Wynne-Edwards, 2002; Storey et al., 2000). Additionally, Berg and Wynne-Edwards (2002) found correlations between mother's and father's cortisol levels and suggested that cortisol might be influenced by chemical signals.

Our previous work on cotton-top tamarin fathers examined both experienced and first-time fathers by measuring urinary prolactin, testosterone, DHT and cortisol over the 6

months of the infants' gestation (Ziegler and Snowdon, 2000). Prolactin levels peaked at midpregnancy for the experienced fathers and the timing of the peak correlated with number of infants surviving from the previous birth. Prolactin levels were higher only in the last month of pregnancy in first-time fathers. Testosterone levels were significantly elevated in both experienced and first-time fathers in the latter half of pregnancy but dihydrotestosterone (DHT) was not. Cortisol levels remained consistent over the gestational period for all males. These results suggested that some reproductive hormones changed before infants' birth but provided no information on the source of the signals the father may be receiving to promote these changes or how these hormones might be effecting other reproductive hormones known to be associated with maternal care behaviors.

The goals of the current study were: (1) to extend our previous work on hormonal changes in parental male tamarins by examining males and females together, (2) to investigate behavioral and hormonal interactions between cotton-top tamarin mates during the entire gestation, (3) to determine if parental experience influenced these interactions and (4) to examine any potential cues from females that would produce hormonal changes in males. Levels of reproductive hormones and behavioral interactions were examined in both expectant mothers and fathers during the entire gestational period. Estrogens aromatized from testosterone influence male parental behavior (Trainor and Marler, 2001, 2002) in California mice. We developed a new urinary steroid separation technique that allowed us to separate and quantify both the androgens and the estrogens in a single sample. In the male cotton-top tamarin, urinary estradiol is derived from gonadal sources but urinary estrone appears to be mainly from extragonadal sources (Ziegler et al., 2000a). In our previous work, we found no change in cortisol levels throughout the pregnancy in expectant fathers using an enzyme assay that measured both urinary cortisol and cortisone. Our present study allowed us to examine additional glucocorticoids that may be responding to signals of pregnancy (Berg and Wynne-Edwards, 2002).

Methods

We monitored 10 groups of captive cotton-top tamarins (*S. oedipus*) during one 6-month gestational period. We considered half of the pairs experienced parents since they had produced at least three successful live births and had multiple offspring living in the family. The other five pairs were considered as less experienced since they either had no previous pregnancies (three pairs) or no more than one surviving infant before the monitored pregnancy (two pairs). All parents had similar experience with sibling births while living in their natal families.

Behavioral data and urine samples were collected three times weekly for all subjects during the 6-month gestation

for both the expectant male and female. The data were collected over a 2-year period as female tamarins became pregnant equally over all seasons between January 1997 and March 1999. There is no seasonality in tamarin reproduction in our captive environment (Ziegler et al., 1987). Housing conditions and husbandry have been described previously for the families in this study (Washabaugh et al., 2002). Lights were kept on a 12:12 h light cycle throughout the year, temperature ranged between 25.6°C and 27.8°C, and since pregnancies occurred at all times of the year, there were no external stimuli changes which could have affected all groups at the same gestational age. Table 1 provides parity history, size of the families at the time of this study and cage space per tamarin for each group. Since larger families had larger cages, we determined the variation in space size per animal. Space per tamarin ranged from 1.00 to 2.4 m³ but there were no significant differences between space per tamarin for experienced and less-experienced families ($t = 0.87$, $P = 0.40$). We adhered to the standards detailed in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Hormonal weeks for each tamarin pair were determined by counting back 184 days from birth. Cotton-top tamarins have a well-defined gestation length of 183.7 ± 1.14 days (Ziegler et al., 1987).

Behavioral methods

Each male–female pair was sampled for 15 min three times a week, resulting in 3 h of observation time per month for each pair. The time of the sessions was alternated each week with 1 week having two morning sessions and one afternoon session and the next week two afternoon sessions and one morning session. Focal animal samples (Martin and Bateson, 1993) were collected on the pair as a unit. A session commenced following a 60-s habituation period. All data were recorded on Tandy Radio Shack TRS-80, Model 100 portable computers. All observers had met predefined criteria of at least 85% (coefficient of concordance, Martin and Bateson, 1993) in interobserver reliability tests before participating in data collection.

Table 1
Tamarin subjects and previous parenting experience

Tamarin pairs	Experience	Parity	# offspring in cage	Tamarin space/m ³
Cait–Nig	experienced	7	5	1.00
Gab–Art	experienced	11	4	1.73
Hon–Tig	experienced	7	3	2.07
Kok–Nic	experienced	4	6	1.58
Van–Did	experienced	7	2	2.44
Phl–Nep	less	1	0	1.75
Val–Jun	less	1	0	1.75
Zep–Wol	less	1	0	1.75
Oct–Squ	less	3	1	1.17
Qui–Yel	less	2	1	1.17

To determine if the pair interactions were changing during the gestational period, we defined seven behavioral categories: proximity maintenance (approach and leave), olfactory signals (scent marking), olfactory investigations (sniffing), sexual investigation (anogenital inspect), sexual/aggressive signals (tongue flick, head shake), sexual interest (mount, attempted mount, male erection), affiliative (groom, huddle, proximity). All behaviors were scored by frequencies except grooming and huddling. At 30-s intervals, proximity was scored on a three-point scale: within arms reach (including contact), within 60 cm and greater than 60 cm. The observations from each week were averaged and then combined into monthly averages for analysis. We excluded analysis of behaviors that occurred at low frequencies for all subjects. Total pair interactions were summed across the gestation period and correlated with number of offspring in the cage and with parity of the pairs by Pearson correlations to see if these variables influenced general pair interactivity. The Hinde Index was used to determine differences between males and females in the maintenance of the relationship (Hinde and Atkinson, 1970). Friedman ANOVAs were used to determine differences in approaches and leaves across the gestation months. We analyzed changes in behavior by months of the gestation for each sex with Friedman tests and post hoc analyses by Wilcoxon signed rank tests. Mann–Whitney U tests were used to compare the experienced fathers (EF) with the less-experienced fathers (LEF) at each month for each behavior category. Behavioral observations were not started on one of the less-experienced pairs (Val–Jun) until the fourth gestational month, although urine was collected throughout the gestation. Therefore, the first 3 months' data for this pair were estimated using an average of other less-experienced pairs weighted by the mean for each month for each behavior. This method has been described (Winer, 1971).

Hormonal measurement

All urine samples were collected freshly as first morning void and frozen at -20°C until sample preparation (Ziegler et al., 1987). Our method for measuring prolactin in cotton-top tamarins has been reported (Ziegler and Snowdon, 2000; Ziegler et al., 1996a,b, 2000a,b). Samples were combined by week with 1.5 ml in duplicate taken for assay. Coefficients of variation for prolactin in this study were inter = 14.37, intra = 6.54 ($n = 15$).

An aliquot (2 ml) of each urine sample used for steroid separation and measurement went through solvolysis (Ziegler et al., 1996b) and solid-phase extraction (Oasis HLB, C18, 60 mg, Waters, Milford MA) before HPLC separation. Solid-phase extraction followed the methods on the instructions except changing the washing step to 20% methanol. The Beckman HPLC equipment and procedure have been described previously (Strier et al., 1999) with the following changes. Samples were injected as 25 μl of acetonitrile/water (50:50) and run isocratic for 40 min at 40/60% acetonitrile/

water. From each sample, fractions were collected for estrone, testosterone and dihydrotestosterone (DHT) for immunoassay analyses (Ziegler et al., 1996a,b, 2000a,b), while estradiol, cortisol, cortisone and corticosterone, which are highly elevated in tamarin samples, were measured directly by UV detection and absorption. UV absorption curves for each standard were linear: cortisol, $R^2 = 0.998$, cortisone, $R^2 = 0.999$, corticosterone $R^2 = 0.9957$, estradiol, $R^2 = 0.997$. Recoveries for the UV assays of added standards were cortisol: 101.33%; cortisone: 80.37%; corticosterone: 94.20%; estradiol: 109.04%. Assay coefficients of variation were: estradiol inter = 12.4 ($n = 17$); estrone inter = 26.2, intra = 6.2, ($n = 7$); testosterone inter = 10.4, intra = 0.51 ($n =$

7); DHT inter = 25.4, intra = 1.5 ($n = 7$); cortisol inter = 7.3 ($n = 12$); cortisone inter = 8.2 ($n = 12$); corticosterone inter = 18.2 ($n = 12$). Cortisol and cortisone were summed to provide a total cortisol excretion for males and females.

All hormones were indexed by creatinine (Ziegler et al., 1995). Hormones were averaged by month, biweekly or weekly for each male and female. Due to different basal hormonal values between the males for androgens (Ziegler et al., 2000a), and prolactin (Ziegler et al., 1996a,b), hormones were expressed as percent change from the first gestational month. Friedman ANOVA was used to determine differences in hormone levels across the gestational months or biweekly with post hoc analyses by Wilcoxon

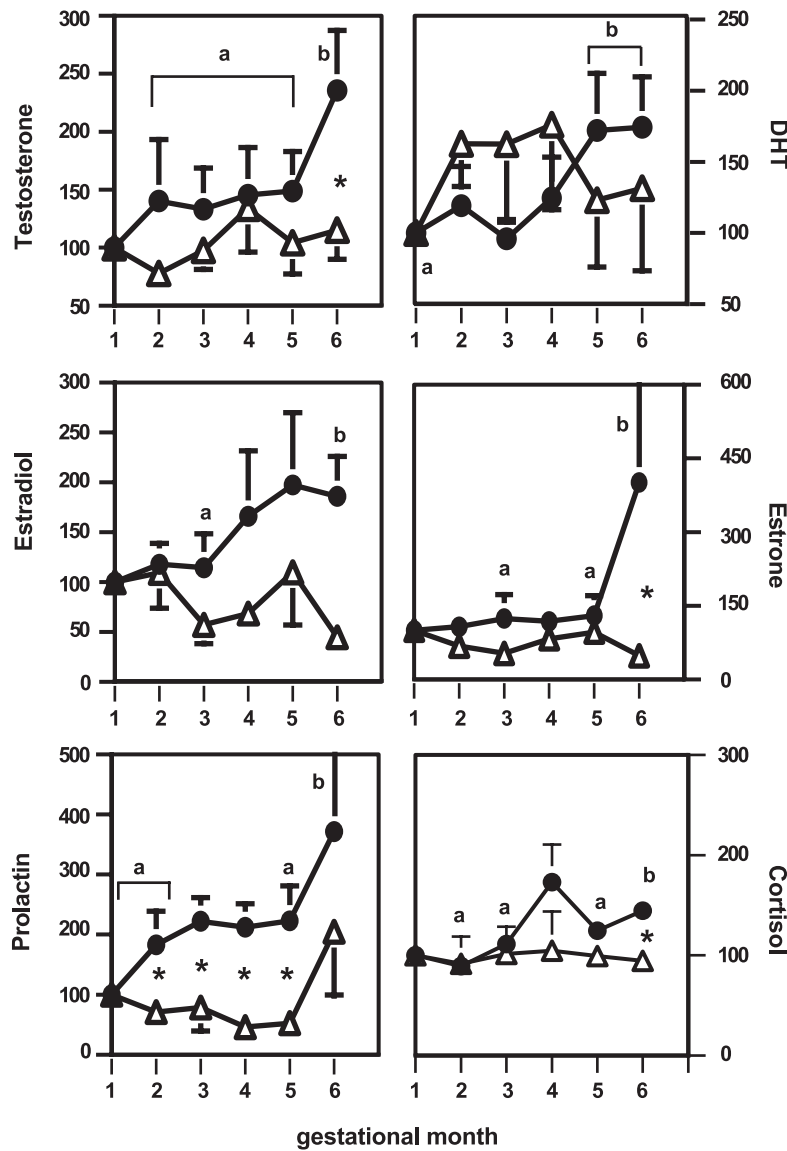


Fig. 1. Mean \pm SEM percent change of urinary hormones from the first month of gestation in 10 expectant father cotton-top tamarins. Month 6 is the last month before birth. Upper left panel: percent change of testosterone; upper right panel: DHT (dihydrotestosterone), mid left panel: estradiol, mid right panel: estrone, lower left panel: prolactin, lower right panel: total cortisol (cortisol + cortisone). Experienced males ($n = 5$) are indicated by the closed circle and less-experienced males ($n = 5$) are indicated by the open triangle. Letters a and b indicate months significantly different from each other for the experienced males. Stars indicate gestation months, which are significantly different between the experienced, and the less-experienced males.

signed rank tests. Hormonal differences by month due to experience (experienced versus less-experienced fathers, using percent change transformed data) were determined by Mann–Whitney U tests. To determine the timing of the increase in cortisol and corticosterone for males and females by week, the first period when the concentration of the glucocorticoid had increased by 2 SD or more from the mean of the previous samples was identified as the period of change. Significance was assessed by P values of less than or equal to 0.05.

Results

Hormonal differences in fathers across gestation and with experience

Experienced males showed both significant changes in hormones across the gestational period and between less-experienced males (Fig. 1). Testosterone, DHT, estrone, estradiol, prolactin and cortisol increased across the gestational months (testosterone: $F = 11.97$, $P = 0.04$; month 6 was higher than months 1, 2, 3, 4 and 5, Z 's > 2.02 , P 's < 0.04 ; DHT: $F = 10.43$, $P = 0.06$; months 5 and 6 were higher than month 1, $Z = 2.02$, $P = 0.04$; estradiol: month 6 was higher than month 3, Z 's > 2.02 , P 's < 0.04 ; estrone: month 6 was higher than months 3 and 5, Z 's > 2.02 , P 's < 0.04 ; prolactin: month 6 was higher than months 1, 2 and 5, Z 's > 2.02 , P 's < 0.05 ; cortisol: $F = 13.46$, $P = 0.02$; month 6 was higher than months 1, 2 and 5, Z 's > 2.02 , P 's < 0.04). Testosterone, estrone, prolactin and cortisol were different from less-experienced males: testosterone: month 6, $U = 25$, $P = 0.009$; estrone: month 6, $U = 24$, $P = 0.02$; prolactin: months 2, 3, 4 and 5 (month 2: $U = 23$, $P = 0.03$; month 3: $U = 23$, $P = 0.03$; month 4: $U = 24$, $P = 0.02$; month 5: $U = 23$, $P = 0.03$); cortisol: month 6, $U = 23$, $P = 0.03$.

Finer point discrimination by 2-week periods showed more variability between experienced males for testosterone, DHT, estradiol, estrone and prolactin, which did not differ significantly across the gestation for the five males. Only the glucocorticoid, total cortisol and corticosterone showed significant changes by 2-week intervals (Fig. 2). Percent change in cortisol varied significantly across the weeks ($F = 23.97$, $P = 0.02$) and weeks 25–26 were significantly higher than weeks 1–14 and weeks 19–22 (Z 's > 2.02 , P 's < 0.04) and weeks 13–14 were significantly higher than weeks 19–22 (Z 's > 2.02 , P 's < 0.04). Percent change in corticosterone levels varied significantly across the weeks ($F = 37.06$, $P = 0.0001$) and weeks 1–10 were significantly lower than weeks 13–16 and 19–20 (Z 's > 2.02 , P 's < 0.04). Weeks 1–6 were significantly lower than weeks 25–26 (Z 's > 2.02 , P 's < 0.04). Average levels for the first 12 weeks of cortisol and corticosterone were significantly lower than weeks 25–26 for cortisol (Z 's > 2.02 , P 's < 0.04) and weeks 13–16, 23–26 for corticosterone ($Z = 2.02$, $P = 0.04$).

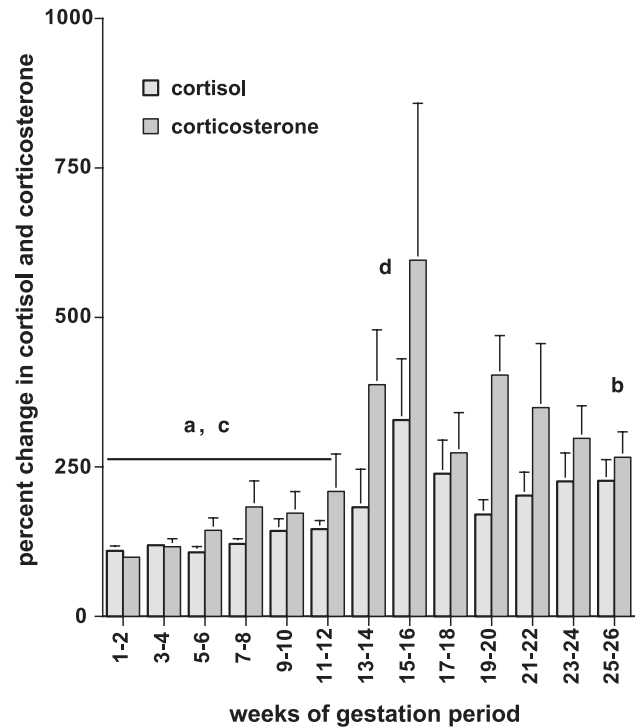


Fig. 2. Mean \pm SEM percent change in glucocorticoid excretion in five expectant experienced fathers. Both total cortisol (cortisol + cortisone) and corticosterone show peak levels during mid gestation. For cortisol, the first 12 weeks of the gestation (a) were significantly lower than weeks 25–26 (b) and weeks 13–14 were significantly different from weeks 19–20. For corticosterone, the first 12 weeks of the gestation (b) were significantly lower than the peak levels at weeks 13–16 (d) and weeks 23–26 (b).

Behavioral interactions in pairs across gestation and with experience

The frequency of total interactions varied considerably between pairs (range 21.71–1503.78) but was not significant by prior parental experience. Total pair interactions were not correlated with number of offspring in the family cage ($r = -0.55$, $P = 0.10$) or parity ($r = -0.46$, $P = 0.18$). The Hinde Index, used to determine who maintains the relationship, revealed nonsignificant differences between males and females over time and no differences as a function of experience. However, all males combined revealed significant differences in the frequencies of their approaches during the last two gestation months ($F = 15.23$, $P = 0.01$; month 5 higher than months 2–4, Z 's > 1.99 , $P = 0.05$). Males had significantly higher amounts of grooming bouts with the female in month 6 than in months 1–5 (Z 's > 2.66 , P 's < 0.01). Proximity between mates increased for the pairs in the last month of pregnancy ($F = 14.46$, $P = 0.01$) and proximity was more frequent in month 6 than months 2–5 (Z 's > 1.98 , P 's < 0.05). Females increased scent marking at the end of the pregnancy ($F = 11.62$, $P < 0.01$; scent marking was higher in month 6 than months 1, 2 and 5, Z 's > 2.09 , P 's < 0.03) while males were grooming their mate more at this time.

Less-experienced pairs showed a higher rate of the following behaviors than experienced pairs. Less-experienced pairs spent significantly more time huddling than experienced pairs (month 5: $U = 2.0, P < 0.03$; month 6: $U = 0, P < 0.01$), more time in proximity with each other (month 3: $U = 1.0, P < 0.02$; month 4: $U = 0, P < 0.01$; month 5: $U = 0, P < 0.01$; month 6: $U = 2.0, P < 0.03$) and the less-experienced females showed more olfactory investigation (month 5: $U = 1.0, P < 0.01$). Less-experienced fathers had a higher amount of mounts (month 2: $U = 2.5, P < 0.03$; month 5: $U = 0, P < 0.01$), erections (month 1: $U = 1.0, P < 0.02$), approaches (month 1: $U = 2.0, P < 0.03$; month 2: $U = 1.0, P < 0.02$; month 3: $U = 1.5, P < 0.02$; month 5: $U = 2.0, P < 0.03$) and leaves (month 1: $U = 2.0, P < 0.03$; month 3: $U = 2.5, P < 0.04$; month 4: $U = 1.0, P < 0.02$; month 5: $U = 2.0, P < 0.03$) than the experienced fathers.

Male and female glucocorticoid response

With one exception, male and female hormones were not coordinated during the pregnancy. We found no significant hormonal correlations between individual pairs for testosterone, DHT, estradiol, estrone or cortisol. However, all pregnant cotton-top tamarin females showed an increase in total glucocorticoid secretion at midgestation. Significant elevations occurred primarily at week 13 for females but varied between weeks 12 and 14. Females showed a sustained increase in glucocorticoids at midpregnancy but no one glucocorticoid, that is, cortisol, cortisone or corticosterone, accounted for this change. Table 2 shows the relationship between the female's rise in glucocorticoids and the male's corresponding increase in cortisol and corticosterone. Each experienced male showed elevated glucocorticoids within 1–2 weeks following his mate's glucocorticoid peak. Only two of the less-experienced fathers showed significant elevations associated with the female's midpregnancy rise.

Table 2
Weekly onset of glucocorticoid increase in expectant mother and father cotton-top tamarin pairs

Pair	Female glucocorticoid	Male corticosterone	Male cortisol	Male total
<i>Experienced</i>				
Ca–Ni	12	13	14	14
Ga–Ar	13	14	14	14
Ho–Ti	13	14	14	14
Ko–Nk	13	14	14	14
Va–Di	13	15	14	16
<i>Less experienced</i>				
Qu–Ye	14	16	14	14
Oc–Sq	13	13	none	none
Ph–Ne	15	none	none	none
Va–Ju	?	none	none	none
Ze–Wo	12	14	13	13

Note: Numbers indicate the first week of a significant hormone elevation more than 2 SD above the mean of the preceding weeks.

Discussion

Male tamarins show prepartum changes in multiple hormones. This study extends our findings of hormonal change to indicate that estrogens and glucocorticoids are changing before parturition in expecting fathers. The cascade of interaction of these hormones is unknown for the tamarin but an increase in androgen production is likely to stimulate estrogen changes. Testosterone's role in paternal behavior appears to be different in mammals than in birds where testosterone is elevated during the breeding season and low during the parenting season (Wingfield et al., 1990). In some monogamous rodents as well as cotton-top tamarins, testosterone may play a role in promoting paternal behavior following aromatization to estradiol (Trainor and Marler, 2001, 2002). The high levels of both androgens and estrogens found in expectant fathers may be explained by an increased production of androgens and subsequent conversion into estrogens. Urinary estradiol is known to have a testicular origin in the male cotton-top tamarin (Ziegler et al., 2000a,b) and both urine and blood levels in expectant fathers are higher than peak levels found for tamarin females during the follicular period of the ovarian cycle (Ziegler, unpublished data and Ziegler et al., 1993). Other New World primates are also known to have elevated blood and urine estrogens (see Brandon et al., 1989, for review). The increased estrogens could promote paternal behavior, as in male California mice (Trainor and Marler, 2002) and may prepare fathers for immediate interaction after infants are born.

Several of the hormones we examined in male cotton-top tamarins are known to promote maternal behavior. In sheep and rats, elevations of estradiol and progesterone are involved in initiating maternal behavior (Bridges, 1996; Kendrick and Keverne, 1991). Studies in female marmosets and tamarins indicate that elevated estrogens are associated with maternal care behaviors (red-bellied tamarins: Pryce et al., 1988; common marmoset: Pryce, 1993; Pryce et al., 1993) but Fite and French (2000) found the opposite for the black tufted-ear marmoset. Maestripieri and Zehr (1998) found a relationship between elevated estradiol and progesterone and maternal behavior in rhesus macaque females. Fleming et al. (1997) found progesterone and estrogens to be related to postpartum feelings of infant attachment in women. However, this relationship between elevated progesterone and estrogens and maternal behavior was not found for the gorilla (Bahr et al., 2001). Less controversial is the role of prolactin in the promotion of maternal care (see Brown, 1993; Buntin, 1996; Dixson and George, 1982; Roberts et al., 2001) and the stimulation of maternal care by oxytocin (Insel, 1990; Kendrick, 2000). Although the proximate mechanisms promoting elevated estrogens and prolactin in biparental males are still unknown and appear to be stimulated differently in females, the end results may be similar.

The pairs increased their proximity to each other during the last 2 months before parturition when the hormones

were most elevated. Males groomed females more often and approached females at a higher rate. While pair interactions were significantly lower in experienced fathers than in less-experienced fathers, all males appeared to be monitoring their females at the end of pregnancy. The hormonal changes occurring in the males might stimulate an increased interest in the female's reproductive state. Experienced males are often involved with interactions with their previous offspring, and therefore may not have the opportunity to monitor the pregnant female as closely as the less-experienced males with few or no offspring. Since experienced males showed the most pronounced hormonal changes and the least pair interactions, experienced males may learn to detect the female's condition without the need to closely monitor the female. Experience affects the response to urinary chemical signals in mice (Nyby and Whitney, 1980).

For the first half of pregnancy in female cotton-top tamarins (first 12 weeks or 90 days), urinary cortisol was excreted at basal levels (Ziegler et al., 1995). During the 13th week, cortisol levels elevated sharply with a sustained elevation throughout the second half of the 26-week pregnancy. Profiles from the current females showed the same profile of sustained glucocorticoid elevation occurring during the 13th week (or 12th week for one female) of gestation and continuing to the end of pregnancy in all females as in our previous study on cortisol. This profile is typical of primates and has been reported for common marmosets (Ziegler and Sousa, 2002), rhesus monkeys (Umezaki et al., 2001), baboons (Pepe et al., 1990), gorillas and chimpanzees (Smith et al., 1999) and humans (Goland et al., 1994; Jaffe et al., 1998). The midgestation rise in glucocorticoids in female primates is attributed to the development of the transitional zone of the fetal adrenal glands, which begins to synthesize cortisol midgestation (Coulter and Jaffe, 1998; Smith et al., 1999; Umezaki et al., 2001) and to the interaction of the fetal–placental–maternal unit. If this occurrence of increased output of glucocorticoids from the maternal–placental–fetal unit is usually followed by a glucocorticoid response in the pair-bonded male, then the rapid fetal–placental–maternal urinary glucocorticoid increase might act as an endocrine signal that initiates a glucocorticoid response in the expectant father.

The rodent literature only focuses on corticosterone and the primate literature only focuses on cortisol, but there is excretion of corticosterone in both nonhuman primates and humans. Corticosterone is not a metabolite of cortisol. The biosynthetic pathway leading to corticosterone in primates is through a direct conversion of progesterone to deoxycorticosterone instead of using the 17-hydroxylase enzyme to produce 17-hydroxyprogesterone. Rhesus monkeys, *Macaca mulatta*, and baboons, *Papio hamadryas*, as well as humans secrete corticosterone into the blood (Goncharova and Lapin, 2002) and, for rhesus monkeys, into the urine as well (Pal, 1979). Both cortisol and corticosterone increase with CRH

administration (Goncharova and Lapin, 2002) indicating a mechanism by which corticosterone could respond to chemical signals via neuroendocrine pathways in the brain. While corticosterone is generally excreted at lower levels than the predominant glucocorticoid, cortisol, during the midgestation peak in expectant male cotton-top tamarins, corticosterone concentrations were significantly higher than cortisol.

Glucocorticoid regulation of pheromones has been found in mice with corticosterone influencing both the release of the signal and the response of the recipient. The pheromones responsible for estrus inhibition in mice (Lee-Boot effect) are regulated by adrenal corticosterone (Drickamer and McIntosh, 1980; Weidong et al., 1998). Urine from intact group-housed female mice can inhibit estrous cycles in singly caged females while urine from adrenalectomized group-housed females does not (Weidong et al., 1998). The replacement of corticosterone to adrenalectomized females restored the ability of urine to inhibit estrous cycles as corticosterone stimulates the release of urinary volatile compounds. Alternatively, a release of corticosterone occurs in singly reared estrous females when exposed to grouped females or their bedding (Marchlewska-Koj and Zacharczuk-Kakietek, 1990). Adrenalectomized mice did not respond to the estrous suppression cues as much as intact mice. An acute increase in plasma corticosterone also occurs in female mice when exposed to male bedding.

The physiological process of enhanced glucocorticoid excretion via the fetal–placental–maternal unit may have evolved into a signaling system for biparental species of nonhuman primates. Anticipation of the pregnancy by the father may work to increase the social bond between the pair, ensuring that the father will remain with the female throughout pregnancy and birth. The glucocorticoid signal may stimulate the onset of other maternal-type hormonal changes in males to ensure his immediate involvement in infants at birth. Female cotton-top tamarins require immediate help in carrying infants due to the large size of the twin infants and the energetic demands of arboreal travelling over large areas to obtain food (Tardif, 1996). Cotton-top tamarin fathers are actively involved in carrying infants shortly after birth (Price, 1991). Therefore, anticipation of the impending birth is important and may ensure a higher infant survival rate. In species such as rodents, marmosets and tamarins, where olfactory communication plays a dominant role in social communication, production of chemical signals during the pregnancy could alert the male to prepare for his role in birthing and parenting.

The urinary glucocorticoids may act to stimulate hormonal changes known to be associated with the onset of maternal behaviors (Bridges, 1996). Some of the less-experienced males appeared less receptive to changes in female urinary cues suggesting that they may need to be learned. However, the increased huddling and grooming seen in less-experienced pairs may also function to prepare the male for infant care. The activation of the fetal adrenal in

cotton-top tamarins appears to induce neuroendocrine changes not only in mothers but also in fathers.

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