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RESEARCH ARTICLE

Changes in Prolactin and Glucocorticoid Levels in Cotton-Top Tamarin Fathers During Their Mate's Pregnancy: The Effect of Infants and Paternal Experience

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We have previously shown that paternally experienced cotton-top tamarin fathers (*Saguinus oedipus*) had significant increases in prolactin and glucocorticoids at the midpoint of their mate's pregnancy, whereas less experienced fathers showed prolactin increases only the month before offspring birth [Ziegler & Snowdon, Hormones & Behavior 38:159–167, 2000; Ziegler et al., Hormones & Behavior 45:84–92, 2004]. These results could be owing to differing paternal experience or from paternal care given to previous offspring. To test the relative role of infant cues and paternal experience in these hormonal changes, we paired four paternally experienced tamarin fathers with a novel, primiparous female and monitored hormone levels during their first pregnancy together. No fathers showed the significant mid-pregnancy increase in prolactin seen previously. However, all fathers showed increases in cortisol and significant peaks of corticosterone in mid-pregnancy. The increase in corticosterone was consistent with previous data occurring in each male during the same week or the week following the urinary cortisol increase shown by his mate. These data may suggest that the elevated mid-gestation prolactin seen previously in experienced males may be owing to the presence of offspring from the previous set of infants. In contrast, increased cortisol and corticosterone occurred independently of infant cues and may be related to previous paternal experience. We therefore conclude that both offspring presence and paternal experience contribute to the hormonal changes seen in experienced cotton-top tamarin fathers during their mate's pregnancy. Am. J. Primatol. 70:1–6, 2008.

Key words: paternal care; hormones; prolactin; cortisol; corticosterone; prepartum; gestation; glucocorticoids

INTRODUCTION

Fathers in bi-parental species can undergo changes in a range of hormones as a result of infant care. One of the best studied of these hormones is prolactin, and it has been shown to be elevated during paternal care in a range of taxa including New World primates [Schradin et al., 2003; Ziegler et al., 1996] and humans [Fleming et al., 2002] and to increase as a direct response to infant contact [Dixson & George, 1982; Mota et al., 2006; Roberts et al., 2001; Ziegler, 2000]. The direction in which the levels of other hormones change during infant care is not as clear cut. For example, males in some non-primate species have been found to have increased cortisol during infant care [Carlson et al., 2006], whereas in primate studies cortisol has been shown to be either reduced [Weid's tufted eared marmosets (*Callithrix kuhlii*); Nunes et al., 2000] or unchanged [cotton-top tamarins (*Saguinus oedipus*); Ziegler et al., 1996], and human fathers have shown increased prolactin after hearing infant cries [Fleming et al., 2002].

In addition to hormonal changes during active infant care, fathers in bi-parental species can also undergo changes during their mate's gestation. For example, Djungarian hamsters (*Phodopus campbelli*), Mongolian gerbils (*Meriones unguiculatus*) and human fathers have shown elevated testosterone before their offspring are born [Berg & Wynne-Edwards, 2001; Brown et al., 1995; Reburn & Wynne-Edwards, 1999, but also see Wynne-Edwards & Timonin, 2007, for arguments for lack of hormonal regulation in bi-parental males]. In female primates, prepartum levels of reproductive hormones and/or cortisol are related to positive infant care [gorillas

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(*Gorilla gorilla*): Bahr et al., 2001; baboons (*Papio hamadryas*) and Japanese macaques (*Macaca fuscata*): Bardi et al., 2003, 2004; humans: Fleming et al., 1997]. These hormonal fluctuations prepartum in fathers are intriguing because males do not undergo the obvious suite of internal physiological and physical changes that mothers do during pregnancy. Therefore, what proximate factors could lead to the hormonal changes observed and what is their causal significance?

Species from the family of Callithricidae are ideal primates in which to ask such questions as they are socially monogamous cooperative breeders where males and females form long-term pair bonds and fathers as well as sub-adult and juvenile helpers contribute to infant care [Yamamoto, 1993]. Cotton-top tamarin fathers carry, groom and retrieve infants from the day of birth and provision infants with solid food during and after weaning [Snowdon & Ziegler, 2007].

We previously reported that cotton-top tamarin fathers undergo changes in many hormones during their mate's pregnancy, including increased prolactin, cortisol, corticosterone, androgens and estrogens, and the pattern of changes differed depending on the father's previous paternal experience [Ziegler et al., 2004]. Paternally experienced fathers (rearing at least three sets of infants) showed significant increases in prolactin, estrogens, androgens and glucocorticoids (cortisol, cortisone and corticosterone) from the mid to the last half of pregnancy. In contrast, fathers who had raised one or no previous sets of infants showed little change in hormones until the last month of pregnancy. All mothers showed sustained elevations in total glucocorticoid secretion starting at mid-gestation [Ziegler et al., 2004] coinciding with the development of the transitional zone in the primate fetal adrenal glands [Coulter & Jaffe, 1998]. All five experienced fathers showed increased levels of both total cortisol (cortisol plus cortisone) and corticosterone within 1–2 weeks of their mate's increase, whereas only three of five less experienced males did so. These results may suggest coordination between hormone excretion in females and their mates. The coordination cues are not yet known, but the inconsistency in hormonal changes in inexperienced fathers suggests a potential role for experience.

Although the extent to which experience influences paternal hormone levels is not yet known, several studies indicate a relationship between these variables. Experienced cotton-top tamarin fathers have higher post-partum prolactin than inexperienced fathers and show a mid-gestational increase of prolactin where inexperienced males do not [Ziegler & Snowdon, 2000; Ziegler et al., 2004]. Additionally, when examining the timing of the prolactin peak as averaged by month, it correlated exactly with the number of infants surviving from the previous birth. The more the surviving infants, the sooner the prolactin peak occurred for the experienced father [Ziegler & Snowdon, 2000]. As females can become pregnant as little as 13 days after giving birth in captivity [Ziegler et al., 1987], experienced fathers are actively caring for infants during their mate's gestation, whereas inexperienced fathers were not.

Our goal was to test the relative importance of infant cues and paternal experience in determining the timing of changes in prolactin, cortisol and corticosterone in experienced cotton-top tamarin fathers during gestation. We tested whether mid-gestational increases in prolactin, cortisol and corticosterone occur in the absence of infant cues by studying paternally experienced fathers paired with a novel, primiparous female. If mid-gestational elevations of prolactin and glucocorticoids are independent of infant cues, then these elevations will appear in experienced fathers without infants. Alternatively, if increased hormonal levels were the result of infant care, experienced fathers without infants would undergo little or no change in these hormones during gestation.

METHODS

Subjects and Experimental Design

We monitored four mated pairs of captive cotton-top tamarins during the first gestation after pairing. Males were all paternally experienced, successfully raising at least four sets of infants with another mate. Each was paired with an unfamiliar nulliparous female and no offspring were present. All parents had helped raise similar numbers of siblings while living in their natal family groups. We collected urine samples twice weekly from both sexes. We began collection immediately after pairing and continued until the infants were born. Cotton-top tamarins have a well-defined gestation length of 183.7 ± 1.14 days [Ziegler et al., 1987]. We calculated conception dates for each pair by counting back 184 days from the day of birth, and counted gestational weeks forward from conception. Females took variable amounts of time to conceive (10–521 days). Housing and husbandry details have been previously described [Washabaugh et al., 2002]. We maintained a 12:12 h light cycle throughout the year and temperature ranged from 25.6 to 27.8°C. We adhered to the NIH Guide for the Care and Use of Laboratory Animals.

Hormonal Measurement

Urine samples were collected as first morning void and frozen at −20°C until sample preparation [Ziegler et al., 1987]. All assays were performed at the Wisconsin National Primate Research Center. Creatinine was measured for all samples by the method reported in Ziegler et al. [1995]. Creatinine intra-assay coefficients of variation for a high and low pool of tamarin urine samples were 3.4 and 2.01%, respectively, and inter-assay coefficients of variation for the same pool were 6.09 and 4.4%, respectively. Prolactin was assayed by radioimmunoassay [Ziegler et al., 2000]. Urine samples were assayed in 1.5 ml duplicates, and intra- and inter-assay coefficients of variation were 12.5 and 14.9%, respectively ($n = 4$).

The glucocorticoids, cortisol plus cortisone and corticosterone were measured in male urine samples by separation of the steroids using high-pressure liquid chromatography and analyzed by ultraviolet detection (UV). Male urine was analyzed for the total cortisol (cortisol plus cortisone, which is a urinary metabolite of cortisol) and corticosterone because these adrenal steroids have been shown to have similar excretions but not the same patterns during the gestational period of the expectant father [Ziegler et al., 2004]. An 1 ml aliquot of urine was put through solvolysis and solid phase extraction (Oasis HLB, C18, 60 mg, Waters, Milford, MA) to purify and concentrate. Samples were injected into the high-pressure liquid chromatography column in 20 μl of 1:1 acetonitrile/water and run isocratic for 30 min at 40:60%. The ultraviolet absorption curves for cortisol, cortisone and corticosterone were linear: $R^2 > 0.99$. Intra-assay coefficients of variation for pooled urine spiked with standards of each hormone were cortisol = 9.23% and corticosterone = 5.86%. Inter-assay coefficients of variation for the same pool were cortisol = 11.38% and corticosterone = 12.08%.

Although male urine samples showed differences in cortisol and corticosterone excretion during the gestational period, the female's samples did not. We therefore measured cortisol in female samples by enzymeimmunoassay to detect the timing of the mid-pregnancy increase without the need of separating the urine for individual glucocorticoids. Antibody cross reactivity was 2.8% for corticosterone, 73.8% for cortisone and 100% for cortisol [Ziegler et al., 1995]. The intra-assay coefficients of variation for a high and low pool of tamarin urine samples were 4.5 and 8.2%, respectively, and inter-assay coefficients of variation for the same pool were 8.4 and 11.8%.

Data Analysis

We used mean weekly hormone levels to determine the timing of the mid-pregnancy cortisol peak in females and the timing of mid-pregnancy increases in cortisol and corticosterone in males. The peak was the first week where the concentration of each hormone increased by at least two standard deviations from the mean of all the previous weeks of the gestational period [see Ziegler et al., 2004]. Samples for males were then averaged by 2-week intervals. Comparisons were made as percent change from peak levels (owing to different excretion amounts between males) by paired *t*-tests for small sample size. Statistical significance was set at $\alpha < 0.05$ and two-tailed tests were used throughout.

RESULTS

None of the males showed a significant increase in prolactin during the mid-gestational period of their mate's pregnancy, but all males showed elevated prolactin at the end of the pregnancy. All but one male showed a significant peak for cortisol, and all males showed a significant peak for corticosterone at or after the time of their mate's onset of cortisol increase (Table I). Two-week values for the males were normalized to the female's cortisol onset in mid-pregnancy as shown in Figure 1. Significant differences were found for male corticosterone levels on the 2-week period of the female's peak from the previous 2 weeks ($t = 3.4$, $P = 0.04$), but no significant differences were found for cortisol or prolactin levels.

DISCUSSION

This study produced three main results. First, none of the fathers showed elevated prolactin in mid-gestation as they had when they were mated to experienced females and had infants present. Second, tamarin fathers exhibited mid-gestation elevations in both cortisol and corticosterone in the absence of infant cues. Finally, the timing of the corticosterone increase was consistent with previous data and occurred in all fathers either during the

TABLE I. Weekly Onset of Glucocorticoid Increase in Expectant Mother and Father Cotton-Top Tamarins (Numbers Indicate the First Week of a Significant Hormone Elevation More Than 2 SD Above the Mean of the Proceeding Weeks)

	Mother	Father		
Pair	Total glucocorticoids	Corticosterone	Cortisol	Total glucocorticoids
Jun–Ani	13	14	16	16
Wol–Net	16	17	13	13
Yelt–Lon	13	14	14	14
Tig–Qui	14	14	13	13

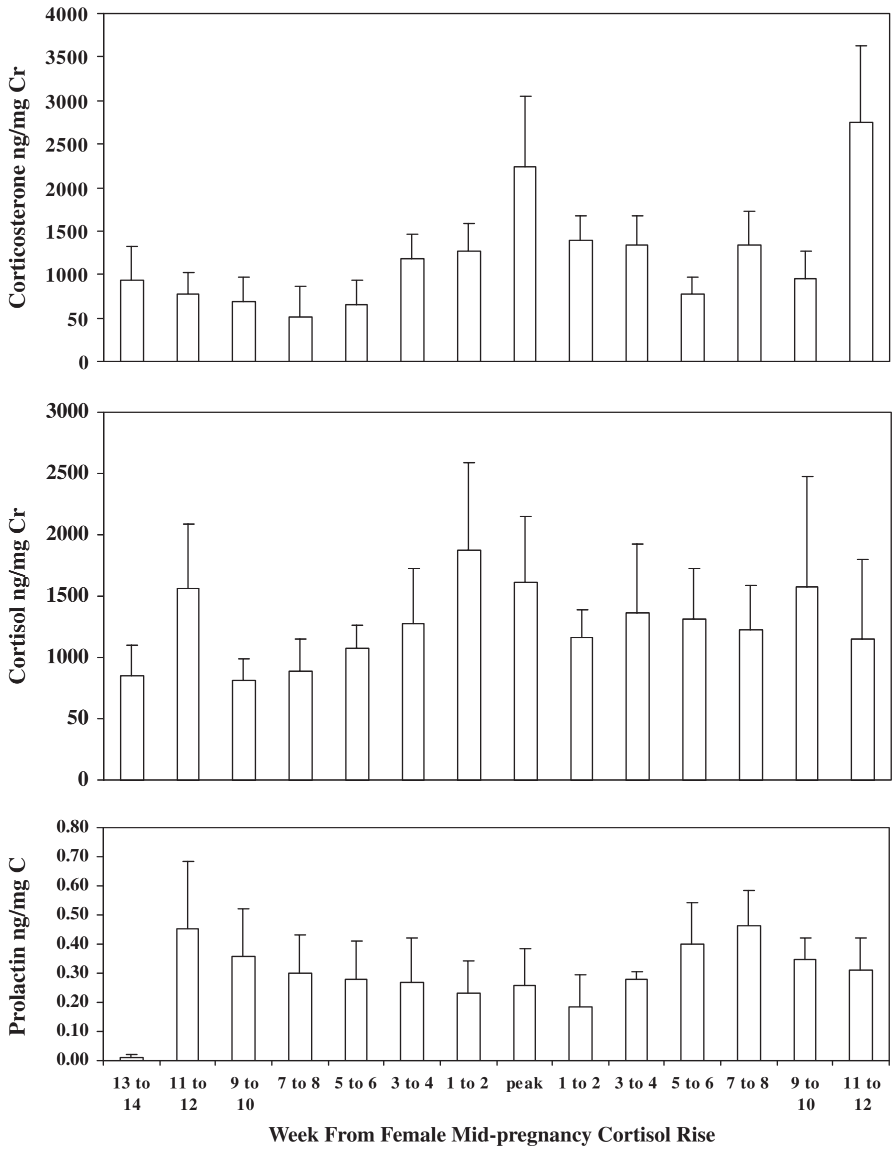

Fig. 1. Mean and SEM levels of corticosterone (upper panel), cortisol (mid panel) and prolactin (lower panel) for expectant father tamarins during their mate's pregnancy. Bi-weekly levels for each hormone were normalized to their mate's onset of cortisol increase.

same week or a week after the mate's cortisol increase.

Prolactin has been reported to increase with infant contact in the cooperative breeding marmoset [e.g. Dixson & George, 1982; Mota et al., 2006; Roberts et al., 2001]. Although we have not shown this in the cotton-top tamarin, we have shown in a previous study that there was a perfect correlation ($r = 1.0$) between the number of surviving offspring from a previous birth and the timing of the prolactin peak in fathers [Ziegler et al., 2000]. This led us to hypothesize that the presence of infants was attributing to the prolactin increase in expectant fathers at mid-gestation. The lack of a mid-gestational increase in prolactin in these males when there were no infants present does support this hypothesis. Infant dependency on carriers in cotton-top tamarins occurs for the first 8 weeks. After this, the infants are more independent and are carried much less. These first 8 weeks would indicate that the offspring would becoming independent while the next gestational period has started if the mates conceive within the first 3 weeks as is normal in our tamarin colony. If prolactin elevation in the experienced fathers were only related to infant contact, then we would expect the highest levels at the beginning of the gestational period. However, it may be the presence of offspring and the family environment that is more important for elevating the male's prolactin. For instance, we have found that males with many offspring in the family do not totally reduce their prolactin levels and that prolactin increases with the number of births [Ziegler et al., 1996]. One male who showed a mid-gestational prolactin increase did not have a new born infant but one that was already 5 months of age when conception occurred for the present pregnancy [Ziegler & Snowdon, 2000]. However, an alternative hypothesis could be that the primiparous females in this study did not provide the same stimuli as do multiparous females. We have found significant differences in the levels of several steroids during pregnancy for experienced females compared with primiparous females, but only during the last trimester of pregnancy [Ziegler & Snowdon, 2004]. At this time, it is not possible to exclude the alternative hypothesis that the lack of a mid-pregnancy prolactin peak in males is owing to differing stimuli from primiparous and mulitparous females.

In contrast, the mid-gestation increases in cortisol and corticosterone can occur without infant cues. Experienced fathers without infants present had mid-gestational glucocorticoids similar to experienced fathers with infants. All fathers showed this increase in corticosterone in the same week as or a week later than the cortisol increase in their mate. We do not know the mechanism for changes in cortisol and corticosterone in fathers during gestation. The changes cannot be due to merely time from pairing since the period from pairing to conception ranged from 10 to 521 days. Ziegler et al. [2004] hypothesized that the increase in corticosterone in fathers was a direct response to the mate's increased cortisol secretion. This study offers additional support because the female's cortisol increase was closely followed by an increase in corticosterone in all four fathers in the study, despite variation in the timing of the females' peaks from 13 to 16 weeks. All primates secrete both adrenal cortisol and corticosterone as was discussed in our previous Ziegler et al., 2004 paper. However, cortisol is the main pathway of glucocorticoid secretion in primates. Both cortisol and corticosterone increase in response to corticotropin-releasing hormone administration in primates [Goncharova & Lapin, 2002] and therefore, both may be receptive to chemical signals from their pregnant mate. As the excretion of corticosterone in male urine is higher at the time of the female's onset of cortisol increase than cortisol in the male urine, it may be involved in the signaling process as is seen in rodents [see Ziegler et al., 2004 for further discussion].

In conclusion, the differences in hormonal profiles of these experienced fathers during gestation from other experienced fathers were the lack of offspring present. Although the mates of these experienced males were not also experienced, the increase in both cortisol and corticosterone occurred but were independent of infant cues. The cues leading to hormonal changes are not yet known, but the apparent coordination between the increase in female urinary cortisol excretion and male corticosterone levels suggests that males respond to cues from pregnant mates. Future research on male responses to endocrine changes in mates and how these lead to changes in male hormones before birth would provide fascinating insights.

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