

Pre- and Postpartum Sex Steroids in Female Marmosets (*Callithrix kuhlii*): Is There a Link with Infant Survivorship and Maternal Behavior?

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Recent studies in primates have suggested that pre- and peripartum sex steroid hormones may be important determinants of maternal behavior and motivation, since higher levels of prepartum estrogen are associated with maternal competency and infant survivorship. To test the cross-species generality of this finding, we monitored excreted profiles of estradiol (E_2), progesterone metabolites (pregnenediol glucuronide; PdG), and E_2 :PdG ratios throughout pregnancy in black tufted-ear marmosets (*Callithrix kuhlii*). Additionally, we wanted to determine the extent to which variability in maternal carrying effort was related to hormonal factors and relative levels of maternal experience. For six females, hormonal profiles were determined by enzyme immunoassay for two pregnancies, one in which infants survived at least 2 weeks postpartum and one in which infants did not survive. Our within-subjects analyses revealed significant differences in mean prepartum E_2 levels for females in the different infant survival conditions. In contrast to previous findings, however, females exhibited significantly *higher* prepartum E_2 levels when their infants did not survive a minimum of 2 weeks postpartum, relative to when their infants did survive. Maternal carrying effort was also negatively and significantly correlated with prepartum E_2 levels. Unlike previous reports in callitrichid primates, then, our data suggest that high concentrations of prepartum E_2 in callitrichid primates are not necessarily associated with competent maternal behavior and may instead be associated with poor infant survivorship and inadequate maternal care. Further, our results appear to be convergent with research focusing on human mothers and may represent a common underlying mechanism linking prepartum estrogen and postpartum affect and behavior in some primates. © 2000

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Mammalian maternal caregiving motivation has been defined as a female's propensity to make an infant the goal of her behavior, resulting in improved infant survivorship and development (Pryce, 1993). Investigations into the proximate mechanisms responsible for the expression of maternal caregiving behavior have led to the identification of two broad classes of determinants of maternal behavior: (1) physiological factors, which include the endocrine changes and sensory stimuli that are associated with pregnancy, parturition, and lactation; and (2) experiential factors, which include experience that is gained through contact with, and providing care to, infants. Decades of research on proximate mechanisms have revealed that the expression of competent maternal behavior is the outcome of a complex interplay between these endogenous and exogenous determinants (see review in Krasnegor and Bridges, 1990).

Among nonprimate mammals, sex steroids and peptide hormones associated with pregnancy, parturition, and lactation are clearly involved in the onset and maintenance of postpartum maternal responsiveness (Bridges, 1990; Poindron and Lévy, 1990). Social experience is also an important factor in the regulation of caregiving behavior among nonprimate mammals. Socialization (Brunelli, Shindledecker, and Hofer, 1989), parturient experience (Bridges, 1975, 1977; Poindron, Lévy, and Krehbiel, 1988), and postnatal experience with infants (Cohen and Bridges, 1981; Poindron *et al.*, 1988), have all been shown to exert positive effects on the expression of maternal caregiving behavior.

Among primates, nonphysiological factors, such as parturient and sibling-rearing experience, have been the predominant focus of past investigations into the

mechanisms regulating the expression of maternal care (see review in Coe, 1990). The importance of parturient experience was demonstrated in a study of rhesus monkeys (*Macaca mulatta*), in which multiparous, but not nulliparous, females exhibited immediate maternal caregiving behavior and attachment when exposed to unfamiliar infants (Holman and Goy, 1980). Another study reported that nulliparous females displayed maternal motivation and caregiving behavior only after parturition (Gibber, 1986). Furthermore, among golden lion tamarins (*Leontopithecus rosalia rosalia*), multiparous females exhibit lower levels of rejection and infant abuse than primiparous females (Hoage, 1978). In addition to parturient experience, sibling-rearing experience has also been identified as an important factor regulating maternal caregiving behavior among callitrichid primates. Epplé (1975) reported that saddleback tamarins (*Saguinus fuscicollis*) with experience rearing siblings exhibit more competent parental care, as well as higher levels of infant survivorship, than parents without sibling-rearing experience. In fact, parturient and sibling-rearing experience has been shown to positively impact infant survivorship in a variety of callitrichid primate species (Kirkwood, Epstein, and Terlecki, 1983; Tardif, Richter, and Carson, 1984; French, Pissinatti, and Coimbra-Filho, 1996b). In each of these studies, however, no direct measure of maternal caregiving behavior was made. Instead infant survivorship was utilized as an indicator of the quality of parental care. Empirical evidence suggests, therefore, that a lack of parturient or infant rearing experience may result in decreased infant survivorship, such that survivorship is mediated by experience-based variation in the quality of maternal behavior.

Although nonphysiological factors have been the predominant focus of past investigations into the mechanisms regulating the expression of primate maternal care, recent studies have implicated hormonal factors in the regulation of caregiving behavior. In group-living pigtail macaques (*M. nemestrina*), females' interest in infants other than their own increased during pregnancy and lactation (Maestripiéri and Wallen, 1995). In nulliparous female macaques, intracerebroventricular injection of oxytocin resulted in increased interest in, and contact with, infants (Holman and Goy, 1995). Among western lowland gorillas (*Gorilla gorilla gorilla*), females demonstrating the greatest maternal competency also exhibited the highest prepartum levels of the urinary progesterone metabolite pregnanediol

glucuronide (PdG; Bahr, 1995). In a study of primiparous human females, mothers with high feelings of attachment toward their infants during the early postpartum period had lower estradiol (E_2) levels and lower E_2 :PdG ratios during early pregnancy than mothers with low attachment (Fleming, *et al.*, 1997a). Moreover, the E_2 levels and E_2 :PdG ratios of females with high attachment feelings showed less of a decline across pregnancy (or no change at all) than did the E_2 levels and E_2 :PdG ratios of females with low attachment. In addition to priming females for the onset of postpartum maternal behavior, endocrine changes also appear to facilitate the maintenance of maternal motivation. Among primiparous human females, postpartum baseline salivary cortisol levels are positively correlated with attraction to infant odors and affectionate contact with infants (Fleming, Steiner, and Corter, 1997b). Among multiparous females, however, postpartum cortisol levels are positively correlated with caregiving behaviors, as well as with postpartum anxiety. These studies suggest that endocrine changes associated with pregnancy may not only prime females for the expression and onset of maternal behavior, but may also function in the maintenance of maternal motivation.

Among callitrichid primates, there is growing evidence that prepartum hormonal status has an important impact on maternal behavior. Prepartum levels of urinary E_2 were significantly higher in female tamarins (*Saguinus labiatus*) that displayed adequate maternal behavior in the first week of infant life, relative to females who displayed inadequate maternal behavior and whose infants died of neglect during the first week of life (Pryce *et al.*, 1988). In an operant setting, pregnant female common marmosets (*Callithrix jacchus*) displayed the greatest level of operant responses to infant stimuli during the time of maximal plasma E_2 :P ratios, immediately prior to birth. Further, treating nonpregnant female marmosets with exogenous E_2 and P in ratios that mimic late-pregnancy concentrations also yielded increased levels of maternally motivated operant behavior (Pryce, Döbeli, and Martin, 1993). The dynamics of endocrine change in late pregnancy may also influence maternal behavior, since primiparous common marmosets that exhibit the greatest urinary E_2 increase and the greatest hydroxypregnanolone decrease during late pregnancy also exhibit the highest levels of maternal motivation (Pryce *et al.*, 1995a). Thus, both correlational and

TABLE 1
Reproductive History of Females

Female	Age (yr)	Condition	Mate	Age (yr)	Length of pairing (mo)	Sibling rearing experience	Number of previous litters	Experienced helpers	Inexperienced helpers	Litter size at birth
An	5	Survived (Re/St)	Ra	7	22	Yes	3	0	2	2
	5	Did not survive	Ra	7	17		2	2	0	1
Ba	4	Survived (In/Ho)	Ca	3	11	No	1	0	1	2
	4	Did not survive	Ca	2	5		1	0	1	1
Bo	3	Survived (Al/Ba)	Hu	8	11	Yes	1	0	1	2
	3	Did not survive	Hu	9	15		2	1	2	2
Co	8	Survived (Ol/Na)	Co	4	1	Yes	1	0	0	2
	8	Did not survive	Co	4	8		2	0	2	2
Lu	3	Survived (Ga/He)	Ki	3	24	Yes	3	0	0	2
	2	Did not survive	Ki	2	10		1	0	0	2
Pi	6	Survived (Dy/El)	He	6	26	Yes	3	0	1	2
	2	Did not survive	He	3	8		0	0	0	3

experimental evidence supports the notion that the prepartum steroidal environment is an important determinant of variation in maternal responsiveness and behavior in callitrichid primates.

The purpose of this study was to test the cross-species generality of endocrine mediation of primate maternal behavior, by examining the relationship between sex steroid hormones, infant survival, and the expression of maternal behavior among female black tufted-ear marmosets, *Callithrix kuhlii*. We wanted to evaluate the hypotheses that prepartum sex steroid concentrations during late pregnancy, and/or postpartum sex steroid concentrations, are associated with maternal behavior and infant survivorship. To evaluate these hypotheses, we contrasted the hormone levels and infant-carrying effort of females, during successful and unsuccessful infant rearing attempts (i.e., whether or not infants survived to 2 weeks of age). Additionally, we examined the relationship between pre- and postpartum steroid concentrations and infant-carrying effort.

METHODS

Subjects

Subjects were six female Wied's black tufted-ear marmosets (*C. kuhlii*) ages 2 to 8 years, housed at the University of Nebraska at Omaha Callitrichid Research Center (see Table 1). The selection criteria for this study were based on female infant-rearing success and reproductive experience. Only females with one

pregnancy in which all infants survived a minimum of 2 weeks postpartum and one pregnancy in which no infants survived were included. In cases in which females had more than one litter meeting these requirements, pregnancies were selected for analysis *a priori* that matched features such as female age, experience, and length of pairing with the mate. Within-subject comparisons of the breeding female and male, and neonates, on demographic and reproductive parameters were made to ensure homogeneity in these measures across different infant survival conditions. Females were housed in family groups, breeding pairs, or two male:one female trios, which had been established 1 to 26 months prior to the respective pregnancy under investigation. All six females were the breeding females of their family or social group. Females were either primiparous (one female (Pi), 1 litter) or multiparous (six females, 11 litters), and all had prior sibling-rearing experience except one (Ba). It should be noted that although this female did not have sibling-rearing experience, she was multiparous for each of the infant-rearing attempts examined in this study.

Marmosets were housed in large cages measuring $1.6 \times 0.9 \times 2.4$ m, which contained natural branches, a feeding platform, a nest tube, and various enrichment devices. Cages containing neighboring groups or pairs were at least 1 m apart and adjoining groups were denied visual, but not auditory or olfactory contact. Our routine husbandry practices and experimental protocols are designed to minimize direct handling

or other disruption of animals, so that animals remained undisturbed throughout the study period. For further details of animal housing and husbandry, see Schaffner *et al.* (1995).

Predictors of Maternal Behavior

Urinary E₂ and PdG profiles, and E₂:PdG ratios, were monitored throughout the reproductive cycle for each female during one pregnancy in which all infants survived a minimum of 2 weeks postpartum and during one pregnancy in which no infants survived. Infants that did not survive for 2 weeks postpartum died an average of 4.00 ± 4.94 (SD) days (maximum 14 days; minimum 1 day) following birth. Additionally, parity was determined for each female, with the number of previous litters for a given female ranging from 0 to 3.

The level of maternal behavior expressed by each female, during the first 2 weeks of infant life, was assessed by quantifying infant-carrying effort. Archival data, in the form of infant-carrying sheets, was used to estimate each female's daily carrying effort. These records are a routine husbandry practice and utilize the scan sampling technique of Altmann (1974). For all new litters born in our colony, a scan sampling check sheet is routinely maintained. At regular intervals throughout the day, the adult or juvenile carrying each infant is systematically recorded. These infant-carrying sheets provide a record of infant caregiving effort and ensure expeditious identification of cases of infant neglect. Most carrying sheet entries (i.e., scans) occurred between 0600 and 1800 h, with an average of 8.12 ± 2.25 (SD) scans per day. From these data, the percentage of scans during which the mother was carrying infants, in the first 2 weeks of infant life, was computed. In cases in which the infants did not survive, data from the scans made prior to the infants' death were used to compute the percentage of scans during which the mother was observed carrying infants.

Procedure

Urine collection. Urine samples were collected five times per week from the six females in this study, as well as from all other animals in our colony, as part of routine colony husbandry. Collecting and archiving urine on a routine basis has proven to be a rich source of information regarding the reproductive and hormonal status of individual animals. A noninvasive

stress-free collection procedure described previously (French *et al.*, 1996a) was utilized. Urine samples were collected between 0600 and 0800 h and centrifuged at 7000 rpm for 2 min to remove detritus, and the supernatant was then transferred to a clean minivial for storage. All samples collected were cataloged and stored at -20°C until assayed. For each female, two samples per week were assayed for each hormone under consideration and their values averaged, providing a weekly estimate of urinary sex steroid excretion.

Estradiol enzyme immunoassay (EIA). An EIA was used to assess the concentration of estradiol-17 β in the urine collected from each female. E₂ has been identified as the major immunoreactive estrogen metabolite for the closely related common marmoset (Pryce *et al.*, 1995b). Urine samples (10 μl) were diluted 1:100 with phosphate-buffered saline and then hydrolyzed with 20 μl of β -glucuronidase/sulfatase (2500 Fishman Units, Type H2; Sigma Chemical, St. Louis, MO). After overnight incubation at 37°C , samples were extracted with 5 ml diethyl ether and reconstituted in 1 ml EIA phosphate-buffered saline (PBS; 0.1 M sodium phosphate, pH 7.0, containing 0.087% NaCl and 0.1% BSA). Extraction efficiency in each assay was monitored by the external recovery of [^3H]E₂ added to extraction tubes and was $94.8 \pm 2.65\%$ ($n = 7$ assays). Sample concentrations were corrected for procedural losses. Microtiter plates (Nunc-Immuno Plate Maxi-Sorp F96) were coated with 50 μl per well of rabbit anti E₂:BSA (diluted 1:15,000 in EIA coating buffer). Cross-reactivities, provided by the Clinical Endocrinology Laboratory at the University of California, Davis, indicated that the E₂ antibody cross-reacted 3.3% with estrone, 0.8% with P, 1.0% with testosterone, and 1.0% with androstenedione. Duplicate 50- μl aliquots of the estradiol standards (Sigma Chemical) ranging from 2500 to 19.5 pg/well ($n = 8$), duplicate 50- μl aliquots of samples (1:100–1:1000 dilution), and E₂-HRP conjugate diluted 1:12,000 in PBS were then added to the plates. After separation of free from bound hormone, ABTS substrate solution (500 μl 40 mM ABTS and 160 μl 0.5 M H₂O₂ added to 49.34 ml 0.05 M citrate buffer, pH 4.0) was added to all wells. Absorbance was measured at 410 nm (reference 570 nm) in a Dynatech MR5000 microplate reader.

The assay sensitivity at 90% binding was 19.5 pg. Displacement curves of halving dilutions of a pregnant female urine pool and E₂ standards were parallel in the 10–90% binding range. Urine samples were diluted to fall in this range. High and low concentra-

tion quality control pools were assayed on each plate, and intra- and interassay coefficients of variation were 3.1 and 10.6% (high, $n = 19$) and 2.9 and 17.3% (low, $n = 19$), respectively.

PdG EIA. Urinary PdG levels were monitored by PdG EIA previously described by Munro *et al.* (1991) and validated for *C. kuhlii* by French *et al.* (1996a). The precision of the assay was determined by assaying high and low concentration quality controls of a mid-pregnancy female urine in each assay run. Intra- and interassay coefficients of variation were 3.2 and 18.3% (high, $n = 23$) and 4.0 and 21.6% (low, $n = 23$), respectively.

Creatinine assay. All hormone concentrations were corrected for the creatinine concentration of each sample. Creatinine concentrations were measured by a modified Jaffé end-point assay (Tietz, 1976) previously described and validated for *C. kuhlii* (French *et al.*, 1996a).

Statistical analyses. In order to ensure within-subject similarity of the breeding female and male, and neonates, on demographic and reproductive parameters, paired-samples *t* tests were conducted. To test the possibility that prepartum steroids are associated with infant survivorship, we compared prepartum sex steroid levels among females in different infant survival conditions. The experiment followed a repeated-measures design. Three completely within-subjects ANOVAs (infant survivorship (2) by weeks (6)) were conducted on the prepartum urinary levels of E_2 , PdG, and the ratio of E_2 :PdG. To test the possibility that infant survivorship is associated with postpartum changes in sex steroid levels, we also compared postpartum levels of these hormones among females in different infant survival conditions. We used completely within-subjects repeated-measures ANOVAs (2×2) to contrast hormone concentrations (and E_2 :PdG ratios) in the 2 weeks after parturition as a function of whether the females' infants survived. Post hoc analyses were conducted using the least significant difference (LSD) test (Cohen and Cohen, 1983). In order to compare the maternal carrying effort of females in different infant survival conditions, we used a paired-samples *t* test. Finally, Pearson correlation coefficients were calculated, to determine whether variation in hormonal concentrations is associated with variation in maternal behavior. An α level of 0.05 was adopted for all statistical tests.

RESULTS

Demographic and Reproductive Parameters

Within-subject comparisons of the breeding female and male, and neonates, on demographic and reproductive parameters indicated a great degree of similarity in these measures across different infant survival conditions. Comparisons of the mean age of females and the number of previous litters for females in surviving and nonsurviving conditions revealed no significant differences ($t(5) = 1.27$, ns, and $t(5) = 1.00$, ns, respectively). Likewise, the age of the breeding male and the length of time each female was paired with her male mate did not differ for the two infant survival conditions ($t(5) = 1.20$, ns, and $t(5) = 1.34$, ns, respectively). Finally, neither litter sizes ($t(5) = 0.54$, ns) nor sex ratios of litters (Fisher's exact $\chi^2 = 1.25$, $df = 1$, ns) differed among the two infant survival conditions.

Prepartum Sex Steroid Profiles

The mean concentrations of urinary E_2 , PdG, and E_2 :PdG across gestation for all females are shown in Fig. 1. Conception occurred 20 weeks prior to parturition, given a gestation length of 143 days (French *et al.*, 1996a). E_2 profiles (Fig. 1A) were characterized by low levels at week -20 prepartum ($33.53 \pm 6.87 \mu\text{g}/\text{mg Cr}$) and rose during the first 6 weeks of gestation. Levels of excreted E_2 were highest from weeks -13 to -6 ($170.03 \pm 10.54 \mu\text{g}/\text{mg Cr}$). A dramatic decrease in E_2 levels occurred at -6 weeks prepartum and levels continued to decrease as parturition approached. PdG profiles (Fig. 1B) were also characterized by low levels at week -20 prepartum ($18.32 \pm 5.11 \mu\text{g}/\text{mg Cr}$) and rose between weeks -20 and -14 prepartum. Urinary PdG reached its highest levels within the first 10 weeks of pregnancy (week -14 prepartum: $41.98 \pm 10.52 \mu\text{g}/\text{mg Cr}$) and steadily decreased as parturition approached. Levels during the last 5 weeks of gestation ($9.41 \pm 0.93 \mu\text{g}/\text{mg Cr}$) were lower than those at week -20. Although there was some individual variability among females, E_2 :PdG ratios (Fig. 1C) tended to be higher later in gestation than during the early phases of gestation. E_2 :PdG ratios declined during the week prior to parturition and in the 2 weeks following parturition.

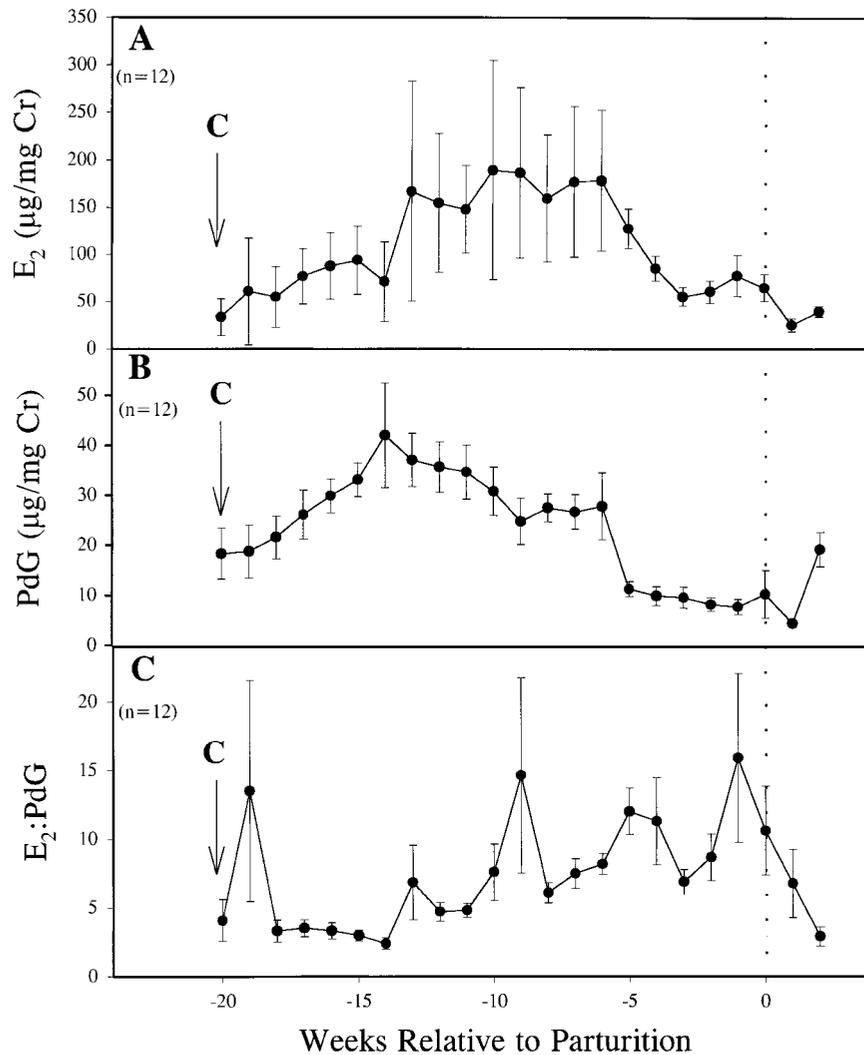


FIG. 1. Mean (\pm SEM) concentrations of excreted urinary E_2 (A), PdG (B), and E_2 :PdG (C) throughout gestation for all females. Data are presented relative to the week of parturition (week 0), indicated by the dotted vertical line. The time of conception is also indicated by the arrow labeled C.

Relationship between Sex Steroid Excretion and Infant Survivorship

Prepartum sex steroid profiles. Urinary E_2 and PdG excretion and E_2 :PdG ratios during the last 5 weeks of gestation are illustrated in Fig. 2. Individual data for each female can also be found in Table 2. With the exception of E_2 concentrations, there were no significant changes in hormone profiles over time during the prepartum phase of the study. E_2 levels declined significantly as parturition approached (LSD, $P < 0.05$), such that concentrations of E_2 were significantly higher -5 weeks prepartum than weeks -3 , -2 , and 0 prepartum (LSD, $P < 0.05$). Concentrations of E_2

were also significantly higher -4 weeks prepartum than weeks -3 and 0 prepartum (LSD, $P < 0.05$).

Prepartum E_2 concentrations differed significantly as a function of infant survivorship ($F(1, 5) = 13.57$, $P = 0.014$). Females exhibited significantly greater prepartum E_2 values when their infants did not survive ($101.70 \pm 8.18 \mu\text{g/mg Cr}$) than when their infants did survive ($55.24 \pm 4.67 \mu\text{g/mg Cr}$). Although neither prepartum PdG concentrations nor E_2 :PdG ratios differed significantly as a function of infant survival, PdG values were slightly higher among females when their infants did not survive ($10.81 \pm 1.20 \mu\text{g/mg Cr}$) than when their infants did survive ($8.02 \pm 0.51 \mu\text{g/mg Cr}$). Additionally, fe-

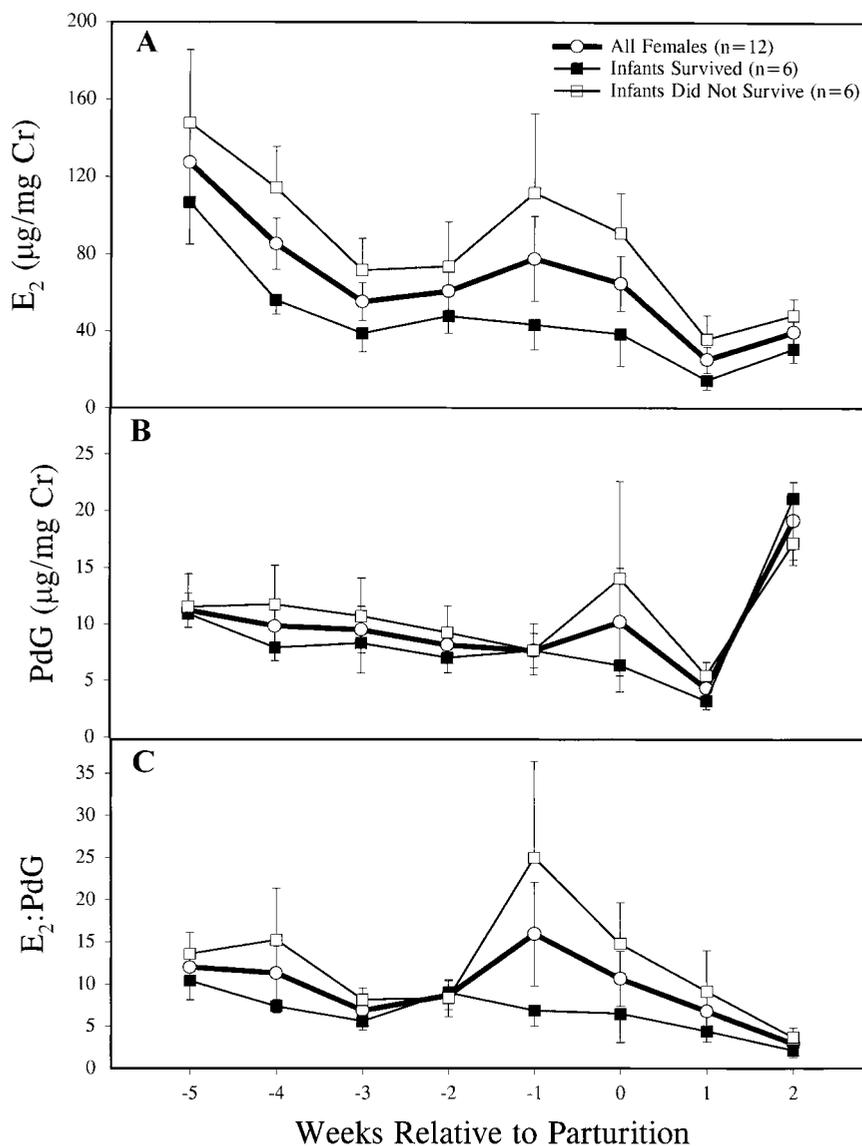


FIG. 2. Mean (\pm SEM) concentrations of excreted urinary E₂ (A), PdG (B), and E₂:PdG (C) from week -5 prepartum through week 0 and weeks +1 through +2 postpartum, for females whose infants did and did not survive. Data are presented relative to the week of parturition (week 0).

male E₂:PdG ratios tended to also be higher when infants did not survive ($14.18 \pm 1.70 \mu\text{g}/\text{mg Cr}$) than when they did survive ($7.62 \pm 0.59 \mu\text{g}/\text{mg Cr}$).

Postpartum sex steroid profiles. The changes in urinary E₂ and PdG excretion and E₂:PdG ratios during the first 2 weeks following parturition are illustrated in Fig. 2 for the females in each infant survival condition. Individual data, by female, are presented in Table 2. Although there were no significant changes in E₂ profiles or E₂:PdG ratios over time

during the postpartum phase of the study, there were significant changes in PdG concentrations. Concentrations of PdG significantly increased between weeks +1 and +2 postpartum (LSD, $P < 0.05$).

Neither postpartum E₂ nor postpartum PdG concentrations, nor E₂:PdG ratios, differed significantly as a function of the infant survivorship category of the mother. Females tended to have greater overall postpartum E₂ values when their infants did not survive ($42.21 \pm 2.98 \mu\text{g}/\text{mg Cr}$), for week +1 through week

TABLE 2
Mean Prepartum and Postpartum Urinary Sex Steroid Levels by Female and Infant Survival Condition

	E ₂ (μg/mgCr)		PdG (μg/mg Cr)		E ₂ :PdG (μg/mg Cr)	
	Infants survived	Infants did not survive	Infants survived	Infants did not survive	Infants survived	Infants did not survive
Female						
An						
Prepartum	69.27 ± 25.46	128.10 ± 15.33	5.97 ± 1.28	10.39 ± 2.90	10.55 ± 2.37	24.08 ± 10.81
Postpartum	21.78 ± 11.73	58.15 ± 3.35	15.08 ± 10.20	11.46 ± 5.41	3.63 ± 3.23	6.36 ± 2.71
Ba						
Prepartum	26.52 ± 6.32	43.63 ± 7.80	4.84 ± 0.49	3.88 ± 0.34	5.24 ± 0.93	11.38 ± 1.83
Postpartum	11.86 ± 5.94	13.20 ± 8.79	8.36 ± 6.98	6.43 ± 4.21	2.72 ± 1.56	2.02 ± 0.04
Bo						
Prepartum	62.73 ± 16.70	62.57 ± 10.03	5.75 ± 1.20	5.74 ± 0.73	11.31 ± 2.12	10.85 ± 1.15
Postpartum	32.94 ± 10.22	36.40 ± 10.13	15.22 ± 12.57	6.80 ± 2.34	5.06 ± 3.51	5.49 ± 0.40
Co						
Prepartum	35.68 ± 6.46	115.49 ± 45.47	7.47 ± 1.42	4.95 ± 0.52	4.86 ± 0.40	22.13 ± 7.54
Postpartum	16.29 ± 5.35	78.89 ± 7.76	3.92 ± 1.81	5.64 ± 2.98	4.48 ± 0.70	20.40 ± 12.16
Lu						
Prepartum	92.98 ± 12.93	151.88 ± 30.00	9.81 ± 1.35	13.22 ± 1.45	10.21 ± 2.39	11.96 ± 2.82
Postpartum	35.56 ± 21.13	45.73 ± 17.14	8.42 ± 2.44	21.39 ± 13.49	3.27 ± 1.95	2.71 ± 0.91
Pi						
Prepartum	44.23 ± 7.44	108.50 ± 24.18	14.26 ± 1.61	26.67 ± 6.11	3.56 ± 1.05	4.67 ± 1.32
Postpartum	18.70 ± 18.34	20.90 ± 5.51	21.96 ± 19.78	16.17 ± 6.68	0.53 ± 0.36	1.39 ± 0.23
Overall means						
Prepartum	55.24 ± 4.67	101.70 ± 8.18	8.02 ± 0.51	10.81 ± 1.20	7.62 ± 0.59	14.18 ± 1.70
Surv ^a		0.008		ns		ns
Wks ^b		0.042		ns		ns
Surv × Wks ^c		ns		ns		ns
Overall means						
Postpartum	22.85 ± 1.97	42.21 ± 2.98	12.16 ± 1.53	11.32 ± 1.11	3.28 ± 0.33	6.40 ± 1.02
Surv ^a		ns		ns		ns
Wks ^b		ns		0.004		ns
Surv × Wks ^c		ns		ns		ns

^a Surv, main effect for infant survival condition; numerical value indicates significance (*P*) level; ns, not significant.

^b Wks, main effect for weeks prior to parturition.

^c Surv × Wks, interaction between infant survival condition and weeks prior to parturition.

+2, than when their infants did survive (22.85 ± 1.97 μg/mg Cr). Additionally, females also tended to have greater overall postpartum E₂:PdG ratios when their infants did not survive (6.40 ± 1.02 μg/mg Cr) than when their infants did survive (3.28 ± 0.33 μg/mg Cr).

Sex Steroid and Parity Effects on the Expression of Maternal Behavior

Infant survivorship was significantly related to the carrying effort of females. In cases in which infants survived, mothers carried more than four times as often, relative to cases in which infants did

not survive ($t(5) = 4.62$, $P = 0.005$). In order to identify variables that might be associated with observed differences in the carrying effort of females, we conducted correlational analyses among measures of maternal effort (i.e., carrying) and pre- and postpartum steroid values and the number of previous litters. These correlations are shown in Table 3. Correlational analysis indicated that an inverse relationship exists between prepartum sex steroid levels and maternal behavior. Females with higher prepartum E₂ concentrations displayed significantly lower levels of infant-carrying effort ($r = -0.63$, $P < 0.05$). Maternal carrying effort was not, however, correlated significantly with the number of

TABLE 3
Correlations between Pre- and Postpartum Sex Steroids, Parity, and Maternal Carrying Scans

Endocrine and experiential variables	Prepartum <i>r</i>	Postpartum <i>r</i>
E ₂	-0.63*	-0.49
PdG	-0.34	0.27
E ₂ :PdG	-0.48	-0.31
Parity		0.31

Note. All *r* values represent the correlation between experiential or endocrine variables and % scans carrying.

**P* < 0.05.

previous litters produced by the female (*r* = 0.31, ns).

DISCUSSION

In contrast to previous reports on callitrichid primates, female tufted-ear marmosets had significantly higher prepartum E₂ levels when their infants did not survive relative to when their infants did survive. When we examined the relationship between sex steroid hormones (both pre- and postpartum) and prior maternal experience, and variation in maternal carrying effort, we found that only prepartum E₂ levels were significantly and negatively correlated with maternal carrying effort.

Recent studies in callitrichid primates have indicated that pre- and peripartum sex steroid hormones may be important determinants of maternal competency, maternal motivation, and infant survivorship (Pryce *et al.*, 1988, 1993, 1995a). In contrast, our data suggest that among *C. kuhlii* females, high concentrations of prepartum reproductive steroids might not necessarily be associated with infant survivorship, but may instead be associated with infant mortality. These results suggest that the relationship between prepartum steroid concentrations and later maternal behavior may apply to a limited set of females and/or may differ across species.

Unlike previous reports, our study adopted a completely within-subjects design, such that each female provided data in both infant survival conditions. By comparing the endocrine profiles of individual females when their infants did and did not survive, we utilized a more efficient and powerful design (Keppel, 1991). Thus, we were able to control for any persistent

trait-like factors that could influence maternal behavior (e.g., Fairbanks, 1996; Maestripieri, Tomaszycki, and Carroll, 1999) and could hence confound the results of a between-subjects design. Our data clearly showed that when females' infants did not survive, their prepartum hormonal profiles were characterized by significantly higher E₂ levels than when these same females' infants survived. Females also tended to have higher overall prepartum PdG levels and E₂:PdG ratios, as well as higher postpartum E₂ levels and E₂:PdG ratios, when their infants did not survive. Thus, based on our findings, it appears that elevated prepartum E₂ production may have been related to infant mortality. Fleming *et al.* (1997a) reported that human mothers with high E₂ levels and high E₂:PdG ratios during pregnancy had more negative mood states during the postpartum period and felt less attached to their infants, providing further evidence for important links between elevated prepartum E₂ and subsequent maternal affect and behavior. Thus, it may be that the differences we observed in maternal carrying effort were modulated by this proposed relationship between postpartum hormones and maternal mood.

To determine whether differences in infant survivorship might be associated with endocrine-mediated variation in maternal behavior, we utilized correlational analyses to determine whether maternal carrying effort was related to endocrine status. Our data indicate that prepartum sex steroids were negatively associated with the carrying effort exhibited by each of the females in our study. In fact, prepartum E₂ levels were negatively and significantly correlated with carrying. The number of previous litters raised by each female, however, was not associated with maternal carrying effort. These results were quite surprising, in light of reports that higher levels of prepartum estrogen are associated with maternal competency and infant survivorship (Pryce *et al.*, 1988, 1993, 1995a; Bahr, 1995).

A remaining question concerns the source and significance of the elevated prepartum estrogen in mothers whose infants succumb prior to weaning. It is well documented that pregnancy is associated with dramatic changes in the mother's hormonal status, as a result of steroids produced by the corpus luteum of pregnancy and the fetoplacental unit (Miller, 1998). The concentrations of these steroids, however, can vary as a function of multiple variables. Fetoplacental factors that might influence placental steroidogenesis include the size of the placenta and the total weight of fetuses, and maternal factors include the length of pregnancy (see discussion in Pryce *et al.*, 1988). Also,

the sex of the fetuses and their health status, as well as maternal age, could all contribute to differences in steroid production and metabolism. Since the females in our study did not differ in age, parity, litter size, or sex ratios in the litters, these factors are not likely to have produced the differences in estrogen concentrations we noted between females in different infant survival conditions. Due to the noninvasive husbandry procedures we adopt in our colony management, we were unable to measure the birth weights or assess the health status of all of the infants in this study or the exact duration of each pregnancy, although all infants did appear to be full term. Thus, although we could not identify the specific source of variation in estrogen production and/or metabolism, we were able to exclude a number of factors.

It remains possible, however, that the elevated sex steroid profiles and decreased levels of carrying we observed in females whose infants did not survive were associated with chronic stress (Pryce *et al.*, 1988). According to this scenario, elevated estradiol and poor infant survivorship both covary with levels of stress. While there is evidence that a variety of stressors can lead to elevated concentrations of sex steroids (MacNiven, de-Catanzaro, and Younglai, 1992; Holzbauer and Newport, 1967), in some cases from adrenal origins (Fajer, Holzbauer, and Newport, 1971), there is also evidence that stressors can lead to lower levels of sex steroids (mice, Wiebold *et al.*, 1986; baboons, Albrecht, Nightingale, and Townsley, 1978). Stress, and the resultant activation of the hypothalamic-pituitary-adrenal axis (HPA), is associated with variation in maternal caregiving behavior in postparturient female gorillas (Bahr *et al.*, 1998) and with responsiveness to infant-related stimuli in human mothers (Fleming *et al.*, 1997b). Pre- and postpartum glucocorticoid levels for female marmosets in different infant survival conditions are currently being documented in our laboratory, and will allow us to determine whether stress or variation in HPA function contributes to variation in infant-rearing success in *C. kuhlii* mothers.

Our finding that prepartum endocrine status, and not parity, was associated with infant survivorship and variation in infant carrying is not surprising in light of reports that parental skills normally gained from experience with siblings can also be acquired through experience with one's own offspring. If females are able to acquire necessary parenting skills through experience with either siblings or their own offspring, then we would expect that the effects of parity may be most evident between primiparous and

multiparous females, without prior sibling-rearing experience. Among common marmosets, primiparous females without sibling-rearing experience exhibited lower levels of infant survivorship than primiparous females with sibling-rearing experience (Tardif *et al.*, 1984). However, no differences in infant survivorship were indicated between multiparous females with and without sibling-rearing experience. In a study of lion tamarins, levels of infant survivorship for multiparous females with and without sibling-rearing experience were not statistically different from those for primiparous females with sibling-rearing experience (French *et al.*, 1996b). Thus, parental skills normally gained through experience with siblings can also be acquired through experience with one's own offspring, although the importance of parity appears to be most evident between primiparous and multiparous females without prior sibling-rearing experience. Given the mean number of prior litters among our females (1.67 ± 0.98), as well as the fact that all but one of our females had sibling-rearing experience, our failure to identify a relationship between parity and carrying might be explained by the differential importance of parity with respect to prior infant-rearing experience.

Among primate and nonprimate mammals, sex steroid and peptide hormones associated with pregnancy, parturition, and lactation are clearly linked to the regulation of maternal caregiving behavior. In addition, perhaps equally important to maternal behavior is prior infant experience. As noted by Pryce (1993), maternal behavior and infant-rearing success are related to a female's sibling-rearing experience, parity, and prepartum endocrine profile, such that the influence of any given factor on maternal behavior is dependent on the level of each of the other factors. The results of this study did not provide support for the facilitation of maternal behavior by high prepartum levels of estrogen. Our findings are particularly exciting, however, as they suggest a highly complex relationship between endocrine status and maternal behavior.

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