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Excretion of urinary steroids in pre- and postpartum female baboons

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Abstract

Steroid hormones are important regulators of a wide variety of reproductive and behavioral functions. We investigated the ability to track sex steroids and glucocorticoids in urine samples collected noninvasively from pre- and postpartum female baboons. Paired plasma and urine samples were collected every 2 weeks prior to and following birth in 10 females. Changes in concentrations of plasma steroids (estradiol, progesterone, and cortisol) were reflected in changes in urinary metabolite excretion (estrone conjugates, pregnanediol conjugates, and cortisol; r's > 0.36, p's < 0.001). A low correlation between prepartum plasma and urinary cortisol may reflect late-gestational changes in the production and/or metabolism of glucocorticoids. Steroid excretion profiles in a large sample of females giving birth and caring for healthy infants ($n = 108$) were compared with profiles obtained from females with poor maternal–fetal outcomes (late-term stillbirth, $n = 14$) and from females with significant postpartum problems with maternal care ($n = 20$). Mothers giving birth to stillborn infants had lower prepartum levels of urinary estrone conjugates and cortisol, suggesting reduced placental steroidogenesis. Mothers with postpartum behavioral difficulties had higher concentrations of prepartum estrone excretion, lower cortisol excretion, and elevated E/P ratios throughout the peripartum period. Noninvasive sample collection and enzyme immunoassay, therefore, have predictive utility regarding circulating steroid concentrations and can identify important endocrine correlates of physiological and behavioral abnormalities in baboons.

Keywords: *Papio*; Baboon; Infant-directed behavior; Hormones; Urinary metabolites; Enzyme immunoassays

1. Introduction

It is becoming increasingly clear that variation in prepartum and postpartum maternal endocrine states in primates is associated with significant individual variation in maternal care and offspring survivorship. For instance, responsiveness to infants among pregnant female macaques varies considerably throughout pregnancy, with increasing infant-directed behavior noted during early and middle pregnancy (Maestripieri and Wallen, 1995) and the highest rates of positive interactions with unrelated infants noted in the third trimester and peaking in the week immediately prior to parturition (Maestripieri and Zehr, 1998). In addition, rates of positive interactions with infants among these females were significantly and positively correlated with plasma estrogen and the ratio of plasma estrogen to plasma progesterone (Maestripieri and Zehr, 1998). In Japanese macaques, maternal responsiveness was related to changing levels of estrogen and cortisol during the late prepartum period (Bardi et al., 2001, 2003). In humans, the ratio of estradiol to progesterone is also associated with variation in women's feeling of attachment to their infants in the immediate postpartum period (Fleming et al., 1997b,a). In callitrichid primates (marmosets and tamarins), there are also links between prepartum endocrine status and maternal behavior and infant survivability. Pryce and colleagues have reported that elevated prepartum estrogens in first-time tamarin mothers are associated with lower rates of postpartum

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infant mortality (Pryce et al., 1988) and that natural and experimental elevation of estrogen to progesterone ratios is associated with high levels of maternal motivation in common marmosets (Pryce et al., 1993; but see also Fite and French, 2000). Thus, at least some of the variation in the quality, quantity, and timing of primate maternal care is regulated by endocrine status.

Endocrine profiles in late pregnancy in female baboons vary dramatically during the last trimester and especially across the days surrounding the parturition itself (Albrecht et al., 1989; Castracane and Goldzieher, 1986; Nguyen et al., 1999). Repeated sampling is crucial in order to accurately monitor these dynamic changes and their potential to modulate contemporary and future maternal responsivity. However, capture and restraint that is required for frequent blood sampling could potentially alter the nature of the sensitive relationship between mothers and their infants, and stress-induced activation of the hypothalamic–pituitary–adrenal axis is a definite possibility with repeated blood sampling. Two solutions to this issue are prominent in the literature. In the first, investigators reduce the frequency of sampling to one or a few samples in any given reproductive state (i.e., late gestation, early prepartum: e.g., Maestripieri and Wallen, 1995; Maestripieri and Zehr, 1998). In the second, repeated samples are collected using noninvasive sampling methodologies for biological substrates other than plasma (e.g., urine, feces: e.g., Bardi et al., 2003, 2004; Fite and French, 2000). These latter techniques are particularly useful only under the condition that there is a clear demonstration that hormone concentrations in the nonplasma biological matrix provides useful information regarding levels of circulating hormones that are affecting the circuits in the central nervous system that regulate maternal care (Numan and Insel, 2003).

The present experiment had two goals: (1) to determine how accurately levels of excreted sex and adrenal steroids and their metabolites in the urine of late-pregnant and early postpartum female baboons reflect variation in circulating levels of these steroids and (2) to characterize normative patterns of urinary hormone excretion in the peripartum in a large sample of females. Ten females in late pregnancy were selected for the first goal, and paired urine and plasma samples were collected at 2-week intervals beginning 9 weeks prior to and following delivery of a live, full-term infant. Plasma estradiol (E_2), progesterone (P_4), and cortisol (pCORT) were measured with radioimmunoassay and urinary estrone conjugates (E_1C), pregnanediol glucuronide (PdG), and cortisol (uCORT) were measured with enzyme immunoassay. To the extent that excreted hormones provide a good proxy for circulating concentrations, we expected to see similar qualitative patterns in circulating and excreted steroids as females undergo the dramatic changes in endocrine status associated with the cessation of pregnancy and the onset of lactation. Further, we anticipated high and positive correlations between plasma and urinary hormones, on all samples collected and within individual females' samples. Finally, endocrine profiles data were derived from 108 females that gave birth to living, healthy, full-term infants. These normative patterns were compared with profiles associated with poor maternal–fetal outcome (stillborn infants) and inadequate postpartum maternal behavior to test whether either of these conditions was associated with endocrine anomalies.

2. Methods

2.1. Animals and study site

We studied female baboons (*Papio hamadryas anubis* sp.) selected from the large colony housed at the Southwest Foundation for Biomedical Research, San Antonio, TX. Adult females were housed in social groups consisting of a single adult-aged male, approximately 30 other adult females, and the infant and juvenile offspring of the adult females. At any point in time, each group contained on average five offspring, ranging in age from newborn to 1.4 years ($M = 0.6$ years old). Offspring were typically weaned and moved to peer groups at about 1 year of age. Adult females sampled in our study ranged between the ages of 5.7 and 19 years, and were identified by numbered tags worn around their necks. All subjects lived in identical large 47-m^2 enclosures. The enclosures contain rocks, ropes, and other climbing structures to provide additional opportunities for activity and enrichment for the baboons. Food and fresh water were available ad libitum, and the feed was supplemented regularly with fresh fruit and grain. Husbandry procedures such as feeding and cleaning occurred on a regular schedule and did not vary between days.

2.2. Paired plasma and urine collection

A subset of 10 females was selected to document patterns of change in concentrations of plasma sex steroids and glucocorticoid hormones in prepartum and postpartum female baboons, and to assess the degree to which these changes were reflected in variation in urinary hormone excretion. The estimated date of conception for each female was determined by the pattern and color of the sex skin swelling, a reliable visual indicator of reproductive status in the baboon (Hendrickx, 1965). The estimated data of full-term delivery was then calculated as 184 days following the date of conception. Urine and blood sampling on females began 9 weeks prior to the expected date of parturition, and continued for 9 weeks after parturition. A single urine and blood

sample once every 2 weeks was collected from each female on the same day. Blood was collected from the saphenous vein of a lightly anesthetized baboon (ketamine and xylazine), stored briefly on ice, centrifuged to separate the plasma, and stored at −20 °C until assayed.

2.3. Excretory profiles in mothers with normal birth, stillborn birth, and postpartum behavioral problems

To establish normative patterns of urinary steroid excretion, urine samples were collected from 108 females housed in similar social and physical conditions as subjects in the first study. The sample included both primiparous and multiparous mothers. Urine was collected from females by allowing them to move from their home enclosure via a transfer chute to a separate smaller individual cage. Females were kept in this area until 5–10 ml of urine was deposited on an aluminum pan located under the individual cage. Using this method required brief (5–30 min) separation of females from their home enclosure, but no handling or restraint was required, and the risk of sample contamination was low. Upon urinating, females were rewarded with fruit and allowed to return to their social group. Attempts to collect urine samples were conducted twice per week for 5 weeks prepartum and 4 weeks postpartum. The females were acclimated to the procedure prior to data collection. The urine was collected via pipette into clean vials, centrifuged briefly to remove detritus, transferred to clean storage containers, and frozen at −20 °C until assay. Sampling occurred at the same time each day for all subjects (between 07:00 and 09:00 h) to minimize variation due to circadian patterns of hormone excretion (Sousa and Ziegler, 1998).

Urine samples collected from two subsets of females were also included in these analyses. First, we analyzed prepartum hormone profiles in females ($n = 14$) who gave birth to stillborn infants. Varying numbers of samples were collected from each female in the prepartum period (range = 1–8). All stillborn infants were late-term, with parturition occurring within 3 weeks of the estimated day of parturition (EDP; range = −21 days prior to the EDP to 4 days following the EDP). For these analyses, we only considered samples collected prior to the date of parturition, to assess whether variation in endocrine profiles was predictive of stillbirth infants. Second, we contrasted endocrine profiles in females ($n = 20$) who delivered normal, full-term infants, but displayed inadequate maternal care that led to the rejection of offspring, or exhibited such poor infant care that a clinical judgment was made to remove the infant because of likely harm due to behavioral neglect and/or abuse. Each female contributed from 4 to 12 samples across the 5 prepartum weeks and 4 postpartum weeks.

2.4. Hormone assays

Radioimmunoassays (RIAs) were used to measure sex and adrenal steroids in plasma samples. Commercial kits from ICN (Costa Mesa, CA) were used to estimate concentrations of estradiol (E_2), progesterone (P_4), and cortisol (pCORT). E_2 and P_4 were measured with double antibody RIAs, while CORT utilized a solid-phase coated tube assay. All RIAs were conducted without sample extraction or chromatography. Each sample was assayed in duplicate, and all samples for each plasma endocrine measure were assayed in a single assay run. Intra-assay coefficients of variation were 2.8, 5.5, and 10.6% for E_2, P_4, and pCORT, respectively.

Previous work on sex steroid metabolism in baboons revealed that estradiol is excreted primarily as estrone and estrone conjugates, while progesterone is metabolized and excreted as pregnanediol conjugates (Wasser et al., 1994). Therefore, sex steroid excretion was measured using enzyme immunoassays (EIAs) for pregnanediol-3-glucuronide (PdG) and estrone conjugates (E_1C). Free cortisol is excreted in the urine of baboons (Ehlers and Killiam, 1980; Yoder et al., 2002) and we utilized a CORT EIA to assess excreted glucocorticoid concentrations. All assays were direct measurement of appropriately diluted urine samples with no extraction or chromatography, and details have been reported elsewhere (Fite and French, 2000; French et al., 1996; Smith and French, 1997). Briefly, microtiter plates (Nunc-Immuno MaxiSorp F96) were coated with 50 μl of antibody raised against a steroid–bovine albumin antigen in rabbit, and diluted in carbonate buffer. Coated plates were sealed, incubated for 1–2 days, and washed prior to remove antibody not covalently bonded to the plate well. EIA buffer was added to each well, along with duplicate aliquots of reference standard (Sigma Chemical), quality control samples, and urine samples, diluted to appropriate concentrations in buffer. Steroid–HRP conjugate was added to wells and the plates were incubated for 2 h. After incubation, the plates were washed to separate unbound from bound hormone. Substrate solution (ABTS-H_2O_2) was added immediately and absorbance was measured at 410 nm (reference 570 nm) with a Dynatech MR5000 Microtiter Plate Reader. A four-parameter sigmoid-fit curve was used to calculate sample concentrations.

Aliquots taken from a pool of pregnant baboon urine were assayed on each plate to monitor assay quality control. The intra-assay coefficients of variation were 3.3, 3.7, and 9.6% for uCORT, PdG, and E_1C, respectively. Interassay CV's were 6.2, 7.5, and 17.5% for uCORT, PdG, and E_1C, respectively. A preliminary assay revealed that serial dilutions of baboon urine from both pregnant and postpartum females produced displacement curves in all assays that were parallel to those produced by the standard curve. To control for

variation in fluid intake and output by baboons, hormone concentrations were corrected for the creatinine content of each sample, using a modified Jaffé end-point reaction assay (described in French et al., 1996).

2.5. Statistical analysis

For the paired plasma–urine phase of the study, mean concentrations of hormone across the pre- and postpartum phases were compared with one-way repeated measures ANOVA's. Because of a large number of missing samples in the first and last sampling periods (9 weeks prepartum and postpartum), analyses were limited to the four samples collected prior to parturition, and the four samples collected after parturition. Significant effects were followed by post hoc dependent-measures *t* tests on successive time blocks, to assess dynamic changes in hormone levels during late pregnancy and the early postpartum period. In addition, the association between variation in urinary and plasma hormone concentrations for each steroid were determined by the use of Pearson correlation analyses. These analyses were conducted across females for all of the paired samples collected during the study, and for each individual female's paired samples.

In the second phase of the study, hormone profiles for normal mothers were established that were based on weekly mean hormone values. Confidence intervals (95%) were calculated for these normative patterns, and mean levels of sex and adrenal steroids for mothers with stillborn infants and mothers with postpartum maternal problems were considered significantly different if they were outside these confidence intervals. In addition, changes across time points for mothers who had uneventful births were also assessed in light of the 95% confidence intervals.

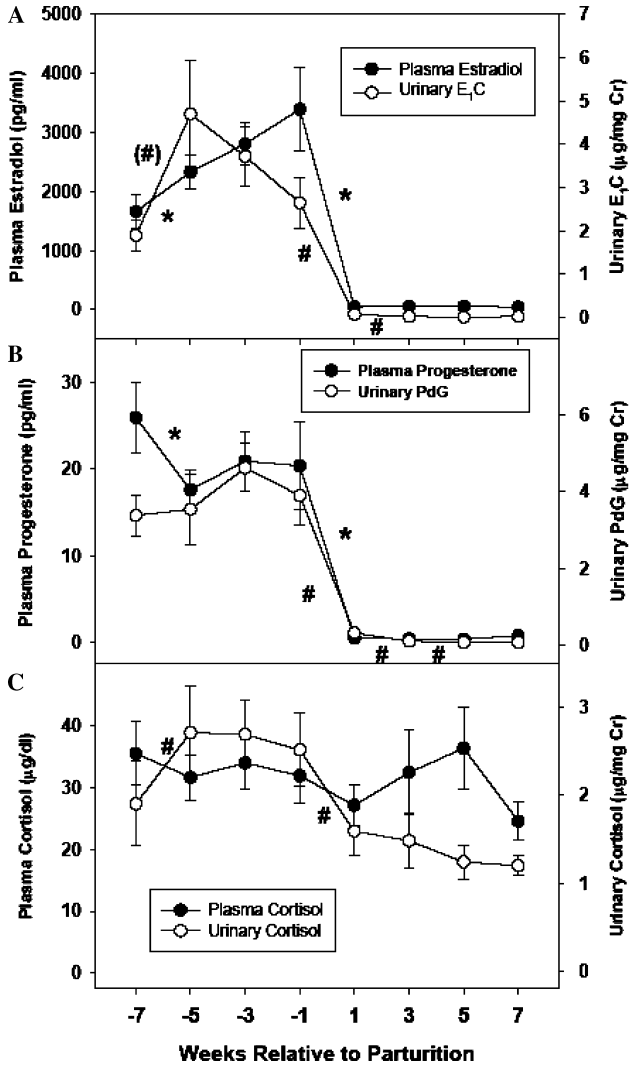

Fig. 1. Mean (±SEM) plasma and urinary hormone concentrations in 10 peripartum female baboons. *, Significant difference in successive plasma hormone levels; #, significant difference in successive urinary hormone levels.

3. Results

3.1. Paired plasma–urinary hormone values

Levels of both plasma and urinary sex steroid changed significantly over reproductive states in female baboons and the patterns of change in both biological substrates were similar (Figs. 1A and B). Plasma E_2 showed significant variation over time ($F(7,63) = 25.57$, $p < 0.001$) with levels rising through late pregnancy (significant increase by post hoc test between Week −7 and −5, $p < 0.023$) and falling dramatically after parturition (Week −1 vs Week +1, $p < 0.001$). These patterns were paralleled by similar significant changes in the excretion of urinary estrogen metabolites ($F(7,63) = 18.19$, $p < 0.001$). Post hoc tests revealed a marginally significant increase in urinary E_1C from Week −7 to −5 ($p = 0.06$), and significant decreases between Week −1 and +1, and Week +1 and +3 (p's < 0.01). Plasma P_4 also varied with pregnancy status in females ($F(7,63) = 19.86$, $p < 0.001$), with levels dropping significantly between Week −7 and −5 ($p < 0.04$) and especially between Week −1 and +1 ($p < 0.001$). As with estrogens, patterns of changes in urinary progesterone metabolites varied across weeks relative to parturition ($F(7,63) = 25.34$, $p < 0.001$). The drop in plasma P_4 from Week −7 to −5 was not noted in urinary PdG concentrations, but the dramatic decrease in urinary PdG from Week −1 to 1 was highly significant ($p < 0.0001$). There was a further decrease from 0.31 ± 0.09 µg/mg Cr in Week +1 to 0.08 ± 0.02 µg/mg Cr in Week +3 and 0.06 ± 0.01 µg/mg Cr in Week +5, differences that were also significant ($p < 0.03$ and $p < 0.05$, respectively).

In contrast to plasma sex steroids, plasma glucocorticoids did not differ significantly across pregnancy

states in female baboons ($F(7, 63) = 1.76$, n.s., Fig. 1C). However, levels of uCORT varied significantly across the weeks immediately prior to and following parturition ($F(7, 63) = 6.77$, $p < 0.001$). Post hoc contrasts revealed that uCORT rose between Week −7 and −5 ($p < 0.01$) and remained elevated in female baboons until parturition, after which levels dropped ($p < 0.02$) and remained low.

Urinary values represent a good estimate of variation in circulating levels of sex and adrenal steroids (Fig. 2).

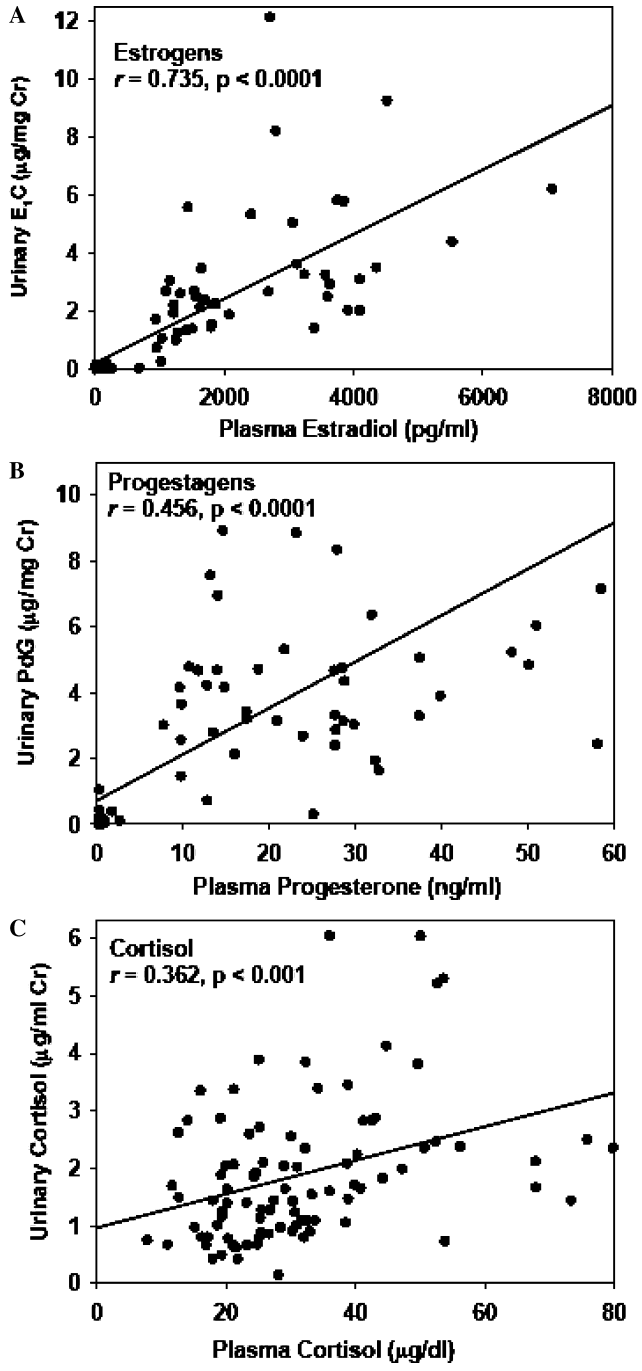

Fig. 2. Scatterplot of all plasma and urinary hormone values for estrogens, progestagens, and cortisol. Line represents best-fit regression.

For all steroid hormones, the correlation between plasma and urinary levels in samples collected on the same day were significant ($p < 0.001$). The relationship was strongest for estrogens ($r = 0.735$), less strong for progestagens ($r = 0.456$), and weakest for glucocorticoids ($r = 0.362$). In the latter case, the relationship accounts for less than 14% of the variance in the plasma and urinary values. The correlation between plasma and urinary cortisol appears to differ, however, as a function of a female's reproductive status. The relationship between plasma and urinary cortisol is not significant when only samples from late-pregnant females are analyzed ($r = 0.29$, n.s.), but plasma and urinary cortisol are significantly correlated when only postpartum samples are included in the analysis ($r = 0.40$, $p < 0.05$).

These differences in sex and adrenal steroids in the correlation between plasma and urinary concentrations are also reflected in patterns of relationships within individual females. Table 1 shows the correlation coefficients for each steroid by female. For sex steroids, all correlations are positive, and 10/10 and 6/10 correlations are significant for estrogens and progestagens, respectively. In contrast, four of 10 correlations between plasma and urinary cortisol are negative, and the paired measures of glucocorticoid activity are significantly and positively correlated only in two of 10 females. The individual analyses also show that the strength of the correlation varies as a function of the pregnancy status of the female. While formal significance testing was not conducted due to small samples sizes (maximum number of paired samples per phase = 5), plasma and urinary cortisol values were more likely to be positive and more likely to be high in the analyses restricted to the postpartum phase, relative to the prepartum correlations.

Fig. 3 presents the steroid hormone profiles during the last month prior to birth and the first month following birth in 108 female baboons with healthy infants and sufficiently good maternal care so that infants are retained for at least the first month of life. The patterns are similar to those observed in the smaller set of females described above. E_1C levels dropped during the last 2 weeks prior to parturition, and exhibit a dramatic and significant decline in the days and weeks following parturition. PdG concentrations are high and stable prior to parturition, and exhibit a 10-fold decline following parturition. Levels of uCORT in the large sample departs from the pattern of cortisol observed in plasma, since again levels drop significantly (nonoverlapping confidence intervals) immediately following parturition and remain low for the first 4 weeks after parturition. The estrogen:progesterone ratio is characterized by high variability, but there appears to be a shift at parturition from a steroid environment more dominated by estrogen to one more dominated by progesterone.

Table 1
Pearson correlations between paired plasma and urine samples collected from pre- and postpartum female baboons ($n = 10$)

Female	n	Estrogens	Progestagens	Cortisol	Cort prepart[a]	Cort postpart[a]
6379	9	**0.91**[b]	**0.90**	−0.21	0.21	−0.32
7724	10	**0.92**	**0.74**	−0.17	−0.07	0.91
7773	9	**0.83**	0.43	0.60	0.41	−0.26
8084	6	**0.88**	**0.77**	**0.98**	0.55	0.59
9881	10	**0.76**	0.61	0.35	0.32	0.51
10425	9	**0.83**	0.46	0.07	−0.43	−0.18
11185	9	**0.93**	**0.86**	−0.01	−0.32	0.13
12373	10	**0.94**	**0.93**	**0.65**	−0.40	0.43
12643	9	**0.81**	0.52	0.61	0.11	0.90
1C0865	10	**0.89**	**0.94**	−0.23	−0.21	0.60
Mean r		0.87	0.72	0.27	0.02	0.33

[a] Correlations calculated on using samples collected during the prepartum or the postpartum phase of the study.
[b] Bold values indicate significant Pearson r between plasma and urinary values; n refers to the number of paired samples per female.

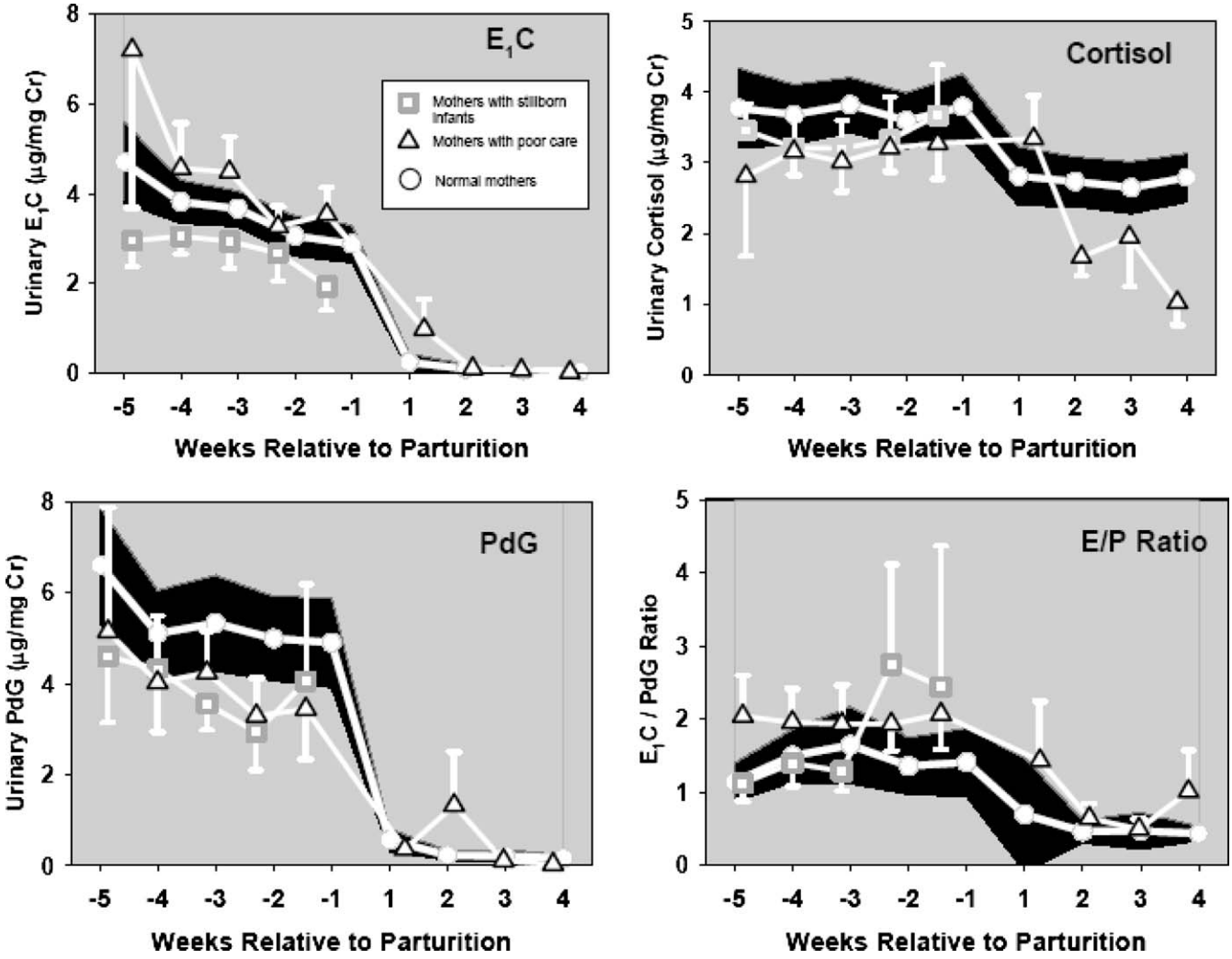

Fig. 3. Mean (±95% confidence intervals) concentrations of urinary steroid hormones in peripartum female baboons with normal postpartum infant care (circles: $n = 108$). Also shown are mean (±SEM) urinary steroid concentrations in female baboons delivering stillborn infants (squares: $n = 14$) and female baboons that displayed inadequate postpartum maternal care (triangles: $n = 20$).

Females who produced stillborn infants or who had significant postpartum behavioral problems with infant care had patterns of steroid excretion that differed from females who had uneventful outcomes (i.e., gave birth to live, full-term infants and showed adequate maternal care). Females with stillborn infants had significantly

lower concentrations of E_1C during all 5 postpartum weeks than normal mothers, and levels of uCORT were significantly lower in weeks −4, −3, and −2 prepartum. Mothers with stillborn infants had significantly elevated E_1C/PdG ratios, especially in the 2 weeks prior to parturition. Mothers that had postpartum behavioral problems with caring for infants, in contrast, had significantly elevated levels of E_1C in 4 of the 5 weeks prior to birth, and in the first week postpartum. For these mothers, levels of excreted PdG were similar to those of normal mothers, with the exception of significantly elevated PdG in the second week postpartum. Levels of excreted CORT tended to be lower for mothers with problems with maternal care both pre- and postpartum, and E/P ratios tended to be higher for these mothers as well, relative to mothers with normal patterns of maternal care.

4. Discussion

Our data show that urinary steroid excretion in pre- and postpartum female baboons provides an accurate assessment of variation in circulating levels of estrogens, progestagens, and glucocorticoids. Analysis of paired urine and plasma samples revealed high correlations between plasma steroids and the excretion of their metabolites in the urine, and the dramatic shifts in circulating sex steroids at parturition were associated with reductions of similar magnitude in excreted sex steroids. Excretion of cortisol closely paralleled changes in circulating levels for postparturient females, but the correlations were less strong for females in the third trimester of pregnancy. Significant differences from profiles of sex and adrenal steroid excretion in normal females were seen in females that produced stillborn infants and in females that experienced significant postpartum difficulties with maternal care. Levels of E_1C and urinary CORT were lower in mothers who produced stillborn infants, and prepartum and immediate postpartum E_1C levels were higher in mothers with significant problems with maternal care.

Initial work on excretion of steroid hormones in female baboons focused on the excretion of estradiol (E_2) and progesterone (P_4; Wasser et al., 1988). While these measures allowed for the identification of significant reproductive events, such as distinguishing follicular from luteal phase and documenting the onset of pregnancy, more detailed analyses of metabolism and excretion of radiolabeled steroids showed that circulating E_2 and P_4 are metabolized prior to excretion. Wasser et al. (1994) infused females with $[^3H]E_2$ or $[^3H]P_4$ and monitored excretion of steroids in urine and feces. E_2 was primarily cleared via the urinary route (90%) and the predominant metabolites were E_1 and E_1 conjugates (64% of total estrogens). Likewise, most P_4 was cleared via the urinary route (60%), and only a small proportion was excreted as P_4, with multiple metabolites in and around the molecular weight of pregnanediol glucuronide. Thus, the antisera we utilized in the present study are immunoreactive with some of the major excretory metabolites of sex steroids in female baboons.

In both phases of the study, parturition was associated with dramatic reductions in urinary concentrations of sex steroids, and these reductions in hormone concentrations reflect the importance of the placenta in regulating hormone concentrations in pregnant baboons. In late pregnancy, steroidogenesis in the female baboon is almost completely dependent upon placental activity (see review in Albrecht and Pepe, 1990). Circulating estradiol in pregnant female baboons appears to be derived primarily from the placental conversion of maternal and fetal adrenal androgens (especially dehydroepiandrosterone and androstenedione; Waddell et al., 1992). Placentally derived estrogen may have important regulatory roles for placental production of progesterone, since treatment of pregnant females with an estrogen antagonist (MER 25) significantly reduces plasma progesterone concentrations (Castracane and Goldzieher, 1986). Further, changes in placental-dependent estradiol biosynthesis may be an important component of the suite of signals for parturition (Albrecht et al., 1989). Finally, circadian changes in circulating estradiol appear to be important cues for organizing normal circadian variation in prolactin production and secretion in the 2 weeks prior to parturition (Nguyen et al., 1999). Given the crucial importance of the placenta, and our demonstrated ability to monitor variations in both estrogen and progestagen metabolites in baboon urine, our noninvasive sampling and assay methodology will prove exceedingly informative to document placental health and function in pathological pregnancies.

We also documented significant endocrine variation associated with two atypical maternal:offspring conditions—stillborn infants and inadequate postpartum maternal care. For the former, we noted that the excretion of estrogens and glucocorticoids were lower in females that produced stillborn infants relative to females that delivered normal-term infants. Experimentally induced reductions in estrogen production, via treatment of pregnant females with GnRH antagonists, is associated with an elevated incidence of stillborn infants (Kang et al., 1989; Siler-Khodr et al., 1984). It appears that monitoring urinary estrogen excretion may be a useful diagnostic tool for assessing risk factors associated with reduced fetal–placental production of estrogens. In the case of glucocorticoids, levels tended to be lower in females at risk for stillborn infants, relative to normal females. Since adrenal steroids appear to be critical precursors or prohormones for placental estrogens in baboons and other primates (Novy and Walsh,

1983; Waddell et al., 1992), variation in adrenal steroidogenesis also appears to be an important diagnostic marker for potential fetal risk.

Finally, a suite of endocrine characters was associated with poor maternal–infant outcomes in the postpartum period derived from insufficiencies in maternal care. These included elevated prepartum and immediate postpartum estrogen excretion, elevated postpartum PdG excretion, low glucocorticoid excretion both prior to and after parturition, and high E_1C:PdG ratios throughout the pre- and postpartum phases. A number of previous studies on nonhuman and human primates have implicated elevated prepartum estrogen as an important primer for later maternal responsiveness (e.g., Maestripieri and Wallen, 1995; Maestripieri and Zehr, 1998; Pryce et al., 1988). Other studies, in contrast, suggest that elevated pre- or postpartum estrogen is associated with reduced maternal responsiveness (e.g., Fite and French, 2000). The results on the present sample of female baboons are straightforward. The reproductive anomalies that lead to the delivery of a nonviable infant are associated with lower rates of estrogen excretion, consistent with the notion that reduced steroidogenesis reflects fetoplacental dysfunction. Behavioral dysfunction, however, is associated with higher production of estrogen. Elevated prepartum and early postpartum estrogen excretion is associated with poor postpartum maternal care, consistent with the findings of Fite and French (2000).Variation in urinary cortisol was less reflective of variation in plasma cortisol, especially in late-term pregnant females. In both phases of the study, females in the late prepartum phase exhibited elevated levels of urinary cortisol, relative to concentrations after the birth of infants. This rise is consistent with reports from other primates, including humans, that pregnancy is associated with a slight hypercortisolemic state, especially in late gestation (see review in McLean and Smith, 1999). In contrast, plasma cortisol did not vary significantly across these same time points. The lack of difference in plasma cortisol between pregnant and nonpregnant females may be associated with an acute stress response associated with the novelty and disturbance of the sample collection procedure for females. Samples were collected from lightly anesthetized female at least 5 min after initiating movement, and the values may represent near-maximal values following a stressor, and thus the values do not represent nonstressed baseline values. In the case of urine, cortisol released in response to handling and anesthesia may not be cleared quickly enough to elevate cortisol, and the bladder in effect integrates values for cortisol over time, resulting in a better measure of overall endocrine setpoints (Whitten et al., 1998). A pregnancy-related change in metabolism of cortisol is also likely to account for the rise in urinary, but not plasma, cortisol. Compared to nonpregnant females, pregnant female baboons in second and third trimester of gestation had a higher proportion of cortisol excreted in an unconjugated form and a lower proportion of cortisol excreted in a conjugated (glucuronide) fraction (Pepe and Townsley, 1975). Our urinary EIA procedure measured free, unconjugated cortisol, whereas the plasma RIA measured total cortisol. Thus, the urinary data do not so much reflect a decrease in the excretion of urinary cortisol after parturition; rather, the elevation in prepartum urinary cortisol is likely attributable to a higher proportion of unconjugated glucocorticoid in samples from third trimester female baboons.

In any event, urinary cortisol in our study was still weakly correlated with variation in plasma cortisol, and may still be useful as an index of activity in the HPA axis. Several observations support this notion. First, initial training for urine collection appears highly stressful for females, and urinary cortisol is significantly reduced with repeated exposure to the protocol. Second, in a subset of samples, urinary cortisol was positively correlated with the time required to collect a sample in the isolation cage ($r = 0.64$, $n = 22$, $p < 0.01$; French and Brent, unpublished data). Finally, urinary cortisol excretion is significantly associated with several measures of maternal care in baboons. Female baboons with higher prepartum cortisol concentrations express more affiliative behavior toward their offspring after parturition, a result that parallels recent findings in both human (Fleming et al., 1997b,a) and nonhuman primates (Bardi et al., 2004; but see Bahr et al., 1998; Bardi et al., 2003). Additionally, postpartum concentrations of cortisol in female baboons are positively correlated with indices of maternal stress (Bardi et al., 2004), and which is similar to reports in other nonhuman primates showing that maternal stress, agitation, and infant rejection are predicted by elevated postpartum cortisol. Thus, while it appears to be important to take into account pregnancy status when assessing urinary cortisol values, the measure appears to be sensitive to physical, social, and behavioral stressors.

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