A Longitudinal Study of Hair Cortisol Concentrations in *Macaca nemestrina* Mothers and Infants

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Abstract

Cortisol is a well-known glucocorticoid that can be used as a biomarker of hypothalamic-pituitary-adrenocortical activity. To explore basal cortisol physiology during pregnancy and infancy in *Macaca nemestrina* monkeys, hair was collected from a convenience sample of 22 healthy mother-infant dyads. Adult females were housed in pairs as part of a small breeding colony at the Washington National Primate Research Center and infants were reared in a specialized nursery. Maternal samples were collected from females during a pregnancy-detection ultrasound and immediately following labor and delivery. Infant samples were collected at birth, 20 days, 4, 6, 8 and 10 mos. of age. Hair cortisol concentrations (HCCs) were determined using an enzyme immunoassay in washed and ground hair samples. Like human mothers, macaque HCCs rose during pregnancy (paired t=5.8, df=16, p<.001). Maternal HCCs at pregnancy-detection (114.2 ± 12.07 picogram/milligram (pg/mg)) were highly predictive of maternal HCCs at delivery (144.8 ± 13.60 pg/mg), suggesting a trait-like quality (r=0.90, P<.001). When maternal HCCs were viewed on a continuum, the absolute rise in cortisol over the course of pregnancy was significantly related to newborn HCCs (r=0.55, P=.02). Infant birth HCCs (1027.4.3 ± 97.95 pg/mg) were seven times higher than maternal HCCs at delivery (paired t = 19.1, df = 16, P<0.001). Higher birth HCCs were strongly associated with larger decreases in infant hair cortisol until 6 months of postnatal age when infant HCCs converged on values indistinguishable from adults. Overall, study results demonstrate a marked degree of fetal cortisol exposure during the latter part of gestation and suggest that the rise in maternal cortisol over pregnancy may play an influential role on HCCs in the newborn.

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Introduction

Glucocorticoids represent a class of steroid hormones that have an array of biological effects and are essential for survival and adaptation [Myers et al., 2014]. Cortisol is the most abundant glucocorticoid and plays a number of vital roles in health that include immune system suppression, maintenance of blood sugar and the metabolism of fat, protein and carbohydrates. In diurnal species, cortisol follows a 24 hour circadian rhythm wherein levels are highest during the morning (approx. 30 min after awakening in humans) and progressively decrease over the day [Corbalan-Tutau et al., 2014]. Environmental stimuli that signal real or perceived threats lead to increased cortisol secretion by the hypothalamic-pituitary-adrenal (HPA) axis, and cortisol concentrations in a variety of biological mediums are used as a surrogate for the measurement of psychological stress and adversity in many species including humans [Weinstock, 2008; Wosu et al., 2013].

The use of hair for the quantitative measurement of cortisol has received increasing recognition in fields such as wildlife biology, ecology, toxicology, forensic science, epidemiology, psychology and medicine over the last several decades [Bévalot et al., 2000; Raul et al., 2004; Kirschbaum et al., 2009; Lafferty et al., 2015; Wester et al., 2015]. The biological mechanisms that allow cortisol to be incorporated into the growing hair shaft are not fully understood, but cortisol present in circulating blood can diffuse into the growing hair shaft and is likely a primary method of incorporation [Meyer & Novak, 2012]. Rather than reflecting cortisol output over hours or days, samples of terminal hair provide a means to measure cortisol activity over the last several months (hair grows an average of about 1cm/mo both in humans and in macaque monkeys) [Dolnick, 1969; Wennig, 2000].

The use of hair cortisol in nonhuman primates has intriguing possibilities for characterizing long-term psychological wellness and evaluating stress management in captive populations [Novak et al., 2013; Carlitz et al., 2014; Baker and Dettmer, 2016]. Like humans, stress in nonhuman primates has been shown to impact HPA activity and the production of cortisol. In rhesus macaques, concentrations of hair cortisol have been shown to be sensitive to stressors such as high population density [Dettmer et al., 2014]. Findings from this study suggest that increased population density is associated with elevated HCCs (hair cortisol concentrations) and that HCC is a viable biomarker of chronic stress in captive primate populations. This hormonal metric has the potential to be a particularly valuable tool for sensitive subgroups such as infants to provide retrospective information about hormone exposure during gestation. A seminal investigation led by Kapoor [Kapoor et al., 2014] demonstrated that hair can successfully be used to measure neuroendocrine function, including cortisol secretion, in pregnant rhesus females and their infants. In the macaque fetus, body hair begins growing around day 90–100 of gestation [Bell, 1969] so that newborn hair reflects cumulative exposure to maternal and fetal derived cortisol across the last two-to-three months of gestation. Neonatal and infant HCCs could potentially be used as a retrospective biomarker of the HPA response to challenges within the prenatal and postnatal environments, including *in utero* exposure to elevated levels of maternal cortisol [Kapoor et al., 2016, Meyer and Hamel, 2014]. Stalder and Kirschbaum [2012] have suggested that fetal exposure to increased concentrations of maternal cortisol should be

considered within the category of relevant environmental exposures that can have enduring effects on health, development and susceptibility to disease.

In this manuscript, we detail a collaborative study between the University of Washington and the University of Massachusetts-Amherst that was designed to provide longitudinal survey data on the normative production of hair cortisol in captive *Macaca nemestrina* mother-infant dyads. Hair was selected to measure cortisol because it can be obtained noninvasively, is easily collected at all age points and is not affected by time of day or current environmental factors. Samples were obtained from a convenience sample of healthy *Macaca nemestrina* monkeys over pregnancy and their nursery-reared infants over the first ten months of postnatal life. Adult females were not subjected to an imposed stressor during pregnancy, so study results provide normative basal hair cortisol values at key physiological time points in this species. Based on the results of previous studies, we hypothesized that maternal HCCs would increase during pregnancy and that newborn HCCs would be higher than maternal levels at delivery. We also hypothesized that there would be a significant positive relationship between maternal and newborn HCCs, illustrating the influence of maternal hormone levels on cortisol deposition in the fetus.

To test our hypotheses, we collected samples to answer the following questions:

> Question #1: How do maternal HCCs change during pregnancy?
>
> Question #2. How do changes in maternal HCCs influence newborn HCCs?
>
> Question #3. How do HCCs change during infancy (over the 1st 10 months of life)?
>
> Question #4. How do newborn HCCs influence the change in HCCs during infancy?

Methods

Subjects:

We initiated an investigation of basal cortisol physiology during pregnancy and infancy in a convenience sample of pregnant *Macaca nemestrina* monkeys and their infants at the University of Washington's National Primate Research Center. From a convenience sample of 22 separate mother-infant dyads, we were able to collect maternal hair samples at pregnancy detection and maternal and newborn hair samples at delivery from 17 mother-newborn dyads to address questions #1 and #2 (maternal–newborn analysis). To address questions #3 and #4, we were able to collect hair samples from 18 infants over the first 10 months of life (infancy analysis). Thirteen infants were included in both the maternal-newborn and the infancy analyses samples (see Table 1). Adult female subjects were pair-housed and regularly provided with fresh fruit, foraging material, treats and enrichment toys in their homecages and monitored by trained staff on a daily basis.

As a convenience sample, the background of the females varied in terms of reproductive history and the level of intervention by human handlers during pregnancy. Nine of the adult females were primiparous and 8 were multiparous. No animal experienced chronic stress during pregnancy but six females were temporarily assigned to a maternal-fetal immunologic study that required preconditioning to a nylon jacket. The length of the jacket

preconditioning varied between animals but typically lasted from several days to two weeks before females were released back to the colony. All subjects were sedated for ultrasound pregnancy detection (approximately 30 days post-conception) and delivery as well as routine veterinary care procedures (e.g. TB testing). The 22 infants in our convenience sample (16 females, 6 males) were hand-raised in a specialized nursery that has developed husbandry procedures to successfully care for young primates [Sackett et al., 2006]. The gestational age ($\bar{x}$=176 ± SE 1.02 days) and birthweight ($\bar{x}$=528 ± SE 13.53 grams) of these infants were normal for the species and illustrate the healthy nature of the pregnancies within this cohort. The mean gestational age and birthweight of infants included in the maternal–newborn analysis and the infancy analysis were nearly identical (see Table 1). All procedures were conducted with the strictest adherence to ethical and regulatory standards set forth by the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Nonhuman Primates and were approved by the Institutional Animal Care and Use Committee at the University of Washington, Seattle, WA, USA.

Hair Collection:

To minimize the disturbance of sampling procedures on pregnant animals, hair samples were collected when the adult females were sedated for routine pre-and postpartum health procedures. This schedule allowed samples to be collected at pregnancy detection and at infant delivery. Hair samples from awake infants were obtained at birth, 20 days and 4, 6, 8 and 10 months.

Sampling Procedure:

Using clippers, the nape of the neck was gently shaved (3 cm by 3 cm) by trained laboratory personnel. The nape was defined as the bottom hair line at the back of the head. Repeated sampling from the same animal was always performed in the same location on the neck (i.e., shave-reshave procedure). Consequently, although the hair sample obtained from each dam at pregnancy detection indexed cortisol accumulation over an indefinite time period prior to shaving (since those samples contained hairs at varying stages in the growth cycle), the second sampling specifically captured cortisol accumulation during the period from pregnancy detection to parturition.

For the infants, the hair sample at birth indexed cortisol accumulation during the period from onset of fetal hair growth to parturition, whereas samples obtained at later time points captured cortisol accumulation during the inter-sampling interval. Samples were tightly wrapped in foil, clearly labeled and placed in a −80°C degree freezer until shipped for analysis. Using express mail, they were then shipped in non-refrigerated batches to the University of Massachusetts at Amherst for cortisol analysis. Cortisol is highly stable in hair and can be stored at room temperature for months, even years, without degradation [Yamanashi et al., 2016].

Cortisol Assay:

The procedures for the hair cortisol assay are detailed in published reports by Meyer et al., 2014 and Davenport et al., 2006. They have been successfully used in a number of published reports on cortisol values in feral and laboratory housed nonhuman primates [Novak et al.,

2014; Dettmer et al., 2009, 2014, 2015]. Briefly, the samples were washed twice with isopropanol to remove external sources of contamination (for example, sweat or sebum) and air-dried. The cleaned hair samples were then powdered by using a ball mill (Retsch, Verder Scientific, Newtown, PA) to break up the hair's protein matrix and increase the surface area for extraction. The entire length of the hair sample was used. Subsequently, approximately 50 mg of powdered hair from each sample was extracted overnight with methanol. Extracts were evaporated, reconstituted in assay buffer, and analyzed in duplicate for cortisol by enzyme immunoassay (Salimetrics, State College, PA). The intraassay coefficient of variation was 1.9% and the interassay coefficient of variation was 6.9%.

Data Analysis:

Data were analyzed using Systat Software (Systat Software, San Jose, CA). HCCs were positively skewed and were log transformed to improve normality of the distributions. The alpha level for reporting significance was $p < 0.05$.

To address Question #1: How do maternal HCCs change during pregnancy, we performed a paired t-test comparing maternal HCCs at pregnancy detection and delivery. The relationship between values at these two time points was assessed by Pearson correlation. Independent t-tests between females subjected to jacket preconditioning and other females, and between primiparous and multiparous females were also conducted to assess any effect of these variables on HCCs at delivery.

To address question #2, how do changes in maternal HCCs influence newborn HCCs, we first performed paired t-tests comparing the newborn HCCs to the maternal HCCs at pregnancy detection and delivery for the 17 animals included in our maternal-newborn analysis. We then performed Pearson correlations to assess the relationships among newborn HCCs and maternal HCCs at pregnancy detection and delivery. We also examined whether the changes in maternal HCCs during pregnancy were related to newborn HCCs using Pearson correlations. We first examined the relationship between newborn HCCs and the percent maternal HCC increase during pregnancy. For this, we calculated the difference between the maternal HCCs at delivery and pregnancy detection. We then divided this difference by the HCC level at pregnancy detection to obtain the percentage increase score. We then examined the relationship between newborn HCCs and the absolute values of the increases in maternal HCCs during pregnancy. For this analysis, we calculated the difference between the maternal HCCs at delivery and pregnancy detection.

To address question #3, how do HCCs change during infancy, we performed a repeated measures ANOVA with infant HCC measures at birth, 20 days, 4 months, 6 months, 8 months and 10 months as repeated measures, and infant sex and maternal parity as between subjects factors to examine differences in HCCs over the first year of life. Post hoc tests for repeated measures were performed using Bonferroni corrections.

To address question #4, how do newborn HCCs influence the change in HCCs during infancy, we derived difference scores for changes in postnatal HCC over the first 10 months of life (birth cortisol minus cortisol at 20 days, 4, 6, 8 or 10 mos.). These absolute difference scores were divided by the infant's birth HCC to obtain percentage change scores for each

age. We then performed Pearson correlations to assess the relationships among newborn HCCs and changes in HCCs during infancy.

Results:

The results of the paired t-test comparing the maternal HCCs at pregnancy detection to HCCs at delivery indicated that there was a significant increase in maternal HCCs during pregnancy (paired t = 5.8, df = 16, P < 0.001). The mean maternal HCC at pregnancy detection was $114.8 \pm$ SE 13.6 picogram/milligram (pg/mg), while the maternal HCC at delivery was $144.8 \pm$ SE 13.60 pg/mg (see Figure 1).

Maternal HCCs at pregnancy detection and delivery were significantly correlated ($r = 0.90$, P< 0.001), suggesting strong intraindividual consistency within cortisol output patterns over pregnancy. Higher levels of HCCs at pregnancy detection were predictive of higher levels at delivery (see Figure 2). There were no differences between adult females with and without jacket preconditioning in maternal delivery samples (t = 0.02, df = 15, P = 0.98). Parity status (multiparous and primiparous) had no impact on maternal HCC values at pregnancy detection (t = 0.2, df = 15, P = 0.82) or delivery (t = 0.2, df = 15, P = 0.87) or on infant HCC values (t = −0.1, df = 15, P = 0.92).

The results of the paired t-test comparing newborn HCCs to maternal HCCs at pregnancy detection and delivery indicated that infant birth HCCs ($1027.4 \pm$ SE 97.05 pg/mg, N = 17) were significantly higher than maternal HCCs at pregnancy detection (paired $t = 18.1$, df = 16, P < 0.001) and delivery (paired $t = 19.1$, df = 16, P < 0.001) (see Figure 1).

The results of the Pearson correlations to assess the relationships among newborn HCCs and maternal HCCs at pregnancy detection and delivery indicated that newborn HCCs were not significantly correlated with maternal HCCs at pregnancy detection ($r = .12$, P = 0.63) or delivery ($r = .33$, P = 0.20). The percent increase in maternal HCCs during pregnancy was moderately, but not significantly, associated with newborn HCCs ($r = 0.45$, P = 0.07). The absolute values of increases in maternal HCCs however, were moderately and significantly correlated with newborn HCCs ($r = 0.55$, P = 0.02) (see Figure 3).

Infant HCCs dropped a precipitous 67% over the first twenty days of postnatal life and stabilized to reach values indistinguishable from adult females (at pregnancy detection) by approximately 6 months of age. By 6 months, infant HCCs averaged $130.4 \pm$ SE 7.4 and represented a mere 15% of birth values. The results of repeated measures ANOVA indicated that the HCCs at birth and 20 days were higher than HCCs at all later time points. HCCs at month 4 were significantly higher than 8 month HCCs and 10 month HCCs (see Figure 4). There was no effect of maternal parity (primiparous vs multiparous; F = 0.3, df = 1,14, P = 0.61) or infant sex (F = 2.2, df = 1,14, P = 0.16) on infant HCCs at birth and throughout the first year of postnatal life.

The results of Pearson correlations to assess relationships among newborn HCCs and percentage change in HCC during infancy indicated that newborn HCCs strongly influenced postnatal patterns of cortisol decline. Infant HCCs at birth were highly correlated with the decrease in infant HCCs over the first six months of life (see Figure 5). This finding suggests

that higher birth HCCs were strongly related to larger decreases in HCCs sampled at 2, 4, 6, 8 and 10 months (see Table 2 for results of Pearson Correlations).

Discussion

In this study, we conducted a longitudinal survey of basal hair cortisol levels in a sample of healthy, *Macaca nemestrina* mother-infant dyads to provide normative values for the species and to explore the relationship between maternal HCCs and cortisol deposition in the infant. Results from this study provide support for our hypotheses on the pattern of cortisol deposition in maternal and fetal hair over pregnancy. Like human mothers, maternal HCCs in this species increase during pregnancy and there is strong intra-individual continuity in cortisol responses to pregnancy that suggest a trait-like quality in long-term HPA activity. Adult females with higher maternal HCCs at pregnancy detection continued to show higher HCCs at delivery. Maternal HCCs at pregnancy detection and delivery were not related to newborn HCCs, birthweight or gestational age. Our results did, however, demonstrate that the absolute rise in maternal cortisol over pregnancy was significantly associated with newborn hair cortisol values. This is the first report in the literature to report such a finding and suggests there may be a strong maternal influence on the deposition of cortisol in fetal hair during gestation.

Consistent with results from a report by Kapoor [Kapoor et al., 2014], newborn HCCs were about seven times higher than that of their mothers. Infant HCCs decreased by 67% over the first twenty days of postnatal life and reached stable values indistinguishable from adult females by 6 months of age. Because birth HCCs showed a wide range of variability, neonatal trajectories of cortisol disposition (i.e., synthesis, degradation and elimination) had to vary widely between subjects to allow convergence on adult HCCs by 6 months of age. This finding suggests that functional maturation of the adrenal cortex in primates may be a highly canalized process [Waddington, 1959; Flatt, 2005]. Our finding of adult-like HCCs by 6 months of age contrasts with results from investigations by Dettmer et al., 2014 and Laudenslager et al., 2012 that showed infant and juvenile macaques have higher HCCs than adults. These inter-study differences may be due to factors such as infant rearing conditions (e.g. nursery, mother and peer), facility differences in colony management and species under study. A combination of these factors may have influenced the results found in this longitudinal study with *Macaca nemestrina* nursery-reared infants and as such, findings may not necessarily apply to macaques reared differently or other species within the Macaca genus.

Much of what is known about the developmental trajectory of cortisol and the HPA axis in primates has been garnered from studies measuring plasma cortisol concentrations. Plasma cortisol concentrations in fetal rhesus monkeys during late gestation (i.e., measured at 130–160 days gestation with an average gestation period of 165 days) are 2- to 3-fold lower than the levels measured in the pregnant dams [Beamer et al., 1972; Kittinger, 1974; Serón-Ferré et al., 1978; Mitchell et al., 1981]. Ontogenic studies of basal HPA function have found stable or slight decreases in blood cortisol concentrations between 2 and 24 weeks of postnatal age [Champoux et al., 1989; Higley et al., 1992; Clarke and Schneider, 1993] with an adult-like pattern of cortisol in place by one year [Sanchez et al., 2005; Barrett et al.,

2009]. The developmental trajectory observed in our study differs from what has been published with plasma and suggests cortisol availability is in place by about 6 months postnatal, significantly earlier than studies using plasma cortisol would suggest. The divergence in results between studies using hair and plasma may reflect the fact that cortisol concentrations vary in different biological mediums (as discussed below) and hair cortisol is not consistently correlated with cortisol obtained from plasma or saliva (Meyer and Novak, 2012).

The existing literature on the developmental biology of the primate adrenal cortex, where cortisol is produced and secreted, has shown that human and nonhuman primates (e.g. macaques, baboons) share strong similarities in the development of the fetal adrenal cortex [Ishimoto and Jaffe, 2011]. The fetal adrenal cortex has two distinct zones, the fetal zone (about 80–90% of the adrenals during gestation) and the definitive zone (widely considered to be the origin of adult adrenal cortices). During late gestation, the fetal zone is distinguished by rapid growth and the production of steroid hormones, including cortisol [Jaffe et al., 1981; Mesiano & Jaffe, 1997]. Enhanced formation of cortisol by the fetal adrenal gland is due to a developmental increase in the expression of 3β-hydroxysteroid dehydrogenase, an obligatory enzyme in the cortisol biosynthetic pathway [Sholl, 1983; Coulter et al., 1996]. Microscopic evaluations of adrenal cortices show a dramatic but orderly remodeling of morphology over the first postnatal year when the thickness of the fetal zone shrinks and the definitive zone grows in size. Adrenal weight dramatically increases during the last 2 weeks of gestation (concomitant with the late surge in fetal cortisol production), declines during the first 2 weeks postpartum due to shrinkage of the fetal zone and then remains relatively stable for a number of succeeding months as the cells within the definitive zone begin to reach structural and functional maturity [McNulty et al., 1981]. The pattern of postnatal infant HCCs described in this paper is consistent with the development of the macaque adrenal gland. Changes in infant adrenal maturation help account for the drop in infant HCCs from 20 days to 2 months, followed by only a slow change in HCCs at later ages tested.

The influence of maternal cortisol on neonatal cortisol concentrations has been somewhat controversial. Early work by Osinski [1960] and later studies by Slikker and colleagues [Slikker et al., 1982, Slikker et al., 1984] demonstrated that the placental barrier enzyme 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2), which converts cortisol to the inactive metabolite cortisone, acts to protect the fetus from exposure to high levels of maternal glucocorticoids. Despite the inactivation of maternal cortisol to cortisone by placental 11β-HSD2, radiotracer studies have shown that approximately 40–60% of fetal cortisol is of maternal origin, [Kittinger, 1974; Mitchell et al., 1981]. These findings suggest that much of the cortisol that can be measured in newborn macaque hair originates from the mother. As cortisol in hair is thought to reflect deposition from the free (unbound) fraction in the bloodstream [Meyer and Novak, 2012], the amount of cortisol binding to plasma proteins, particularly corticosteroid binding globulin (CBG), could also play a role in determining neonatal HCCs. Data from rhesus macaques indicates that plasma cortisol binding to CBG is lower at birth than in adulthood (i.e., a greater percentage of cortisol is unbound in the neonate) but rises over the first 1–2 years of life [Beamer et al., 1973]. Finally, although the infant's own adrenal glands are presumably the primary source of cortisol deposited in hair

from the day-20 time point onward, we note that in mother-reared infants cortisol can be transferred from the lactating dam to her offspring via milk, and that variation in milk cortisol levels has been linked to infant temperament [Sullivan et al., 2011; Hinde et al., 2015]. The amount of cortisol measured either in infant plasma or hair that derives from milk is not yet known.

One important goal of this study was to establish basal HCCs for macaque females and their infants, providing the detail that is necessary to describe the normative expression of hair cortisol over pregnancy and the first postnatal year. The results of this study are in concordance with data reported by Kapoor et al., [2014]. The range of maternal HCCs at delivery in the current study was 76.6 to 245.1 pg/mg with a mean of 144.8 ± 56.1 pg/mg (mean ± SD), while the average maternal HCC in the Kapoor cohort was 130 ± 46.53 pg/mg (mean ± SD) (no ranges provided). Pregnant females were housed in very similar laboratory environments in both studies. Newborns from the current investigation showed a range of 601.4 to 2082.5 pg/mg with a mean birth HCC of 1027.4 ± 400.1 pg/mg (mean ± SD) while the Kapoor cohort showed a mean newborn HCC of 1093 ±340 pg/mg (mean ± SD) (no ranges provided). These two normative biophysiology studies demonstrate that macaques have HCCs of about 1000 pg/mg at birth, values that are dramatically higher than those found in maternal hair samples collected at the same time point.

Several studies by Dettmer and colleagues [Dettmer et al., 2009, 2014, 2015] have examined hair cortisol in adult and infant rhesus macaques to investigate the relationship between HCCs and population density, maternal parity, other hair hormone concentrations and infant neurobehavioral development. A 2009 study by Dettmer et al reported an average HCC of 176.88 ± 13.08 pg/mg (mean ± SD) for six-month old, nursery-reared rhesus infants , a value that did not differ from mother and peer-reared animals of the same age (172.94 ± 12.36 mg/mg) [Dettmer et al., 2009]. Mean HCCs in the current study with 6 month old nursery-reared pigtail macaque infants averaged 137.3± 41.21 (mean ± SD), a value that also suggests nursery-reared animals do not display higher HCCs when compared to same-aged peers raised in more naturalistic rearing environments. While it is tempting to interpret this finding in the context of reduced psychosocial stress, Capitanio and co-authors have pointed out that there may be a generalized muting of the stress response in nursery-reared macaques that makes interpretation of cortisol values difficult [Capitanio et al., 2006]. A pattern of higher HCCs in primiparous dams during late pregnancy/early lactation found in a Dettmer 2015 investigation was not replicated in the current study. This divergence in results is likely due to differences in sample size, species or sampling time points. We also did not find an effect of maternal parity on infant HCCs. This may be the result of the standardized housing that our infants receive in the nursery, eliminating the influence of different care-taking styles that can characterize naïve and experienced macaque mothers.

The results of this longitudinal study provide substantial evidence that hair can be used as a hormone-bearing biological substrate to measure neuroendocrine (specifically adrenocortical) activity in *Macaca nemestrina* mother-infant dyads. The current findings demonstrate a marked degree of fetal cortisol exposure during the latter part of gestation and suggest that the rise in maternal cortisol over pregnancy may play an influential role on HCCs in the fetus. The pattern of hair cortisol decline during infancy suggests that an adult-

like pattern of cortisol availability is in place by about 6 months of postnatal age, earlier than studies using blood cortisol would suggest. The different estimates of cortisol maturation gleaned from hair compared to blood sampling could be partially due to the above mentioned rise in CBG during postnatal development, a factor that would not directly influence plasma cortisol measurements but would presumably reduce the fraction of blood-borne cortisol available for incorporation into hair. The use of hair samples as a hormonal metric for animals and humans could be important for future studies of chronic stress, elevated cortisol output, and altered developmental programming in affected offspring. It also holds promise for exploring the long-term relationship between early hormone profiles and infant neurodevelopmental outcomes. Previous reports with rhesus macaques have reported that HCCs are predictive of early cognitive development [Dettmer et al., 2009} and higher maternal HCCs are associated with adverse changes in infant behavioral state (more irritable, less consolable) and lower sensorimotor reflex scores [Dettmer et al., 2015]. Our future study efforts will focus on increasing the sample size of our mother-infant cohort, expanding our investigation of HCCs in mother-reared infants and exploring the relationship between infant HCCs and measures of cognition, temperament and social development.

Acknowledgements:

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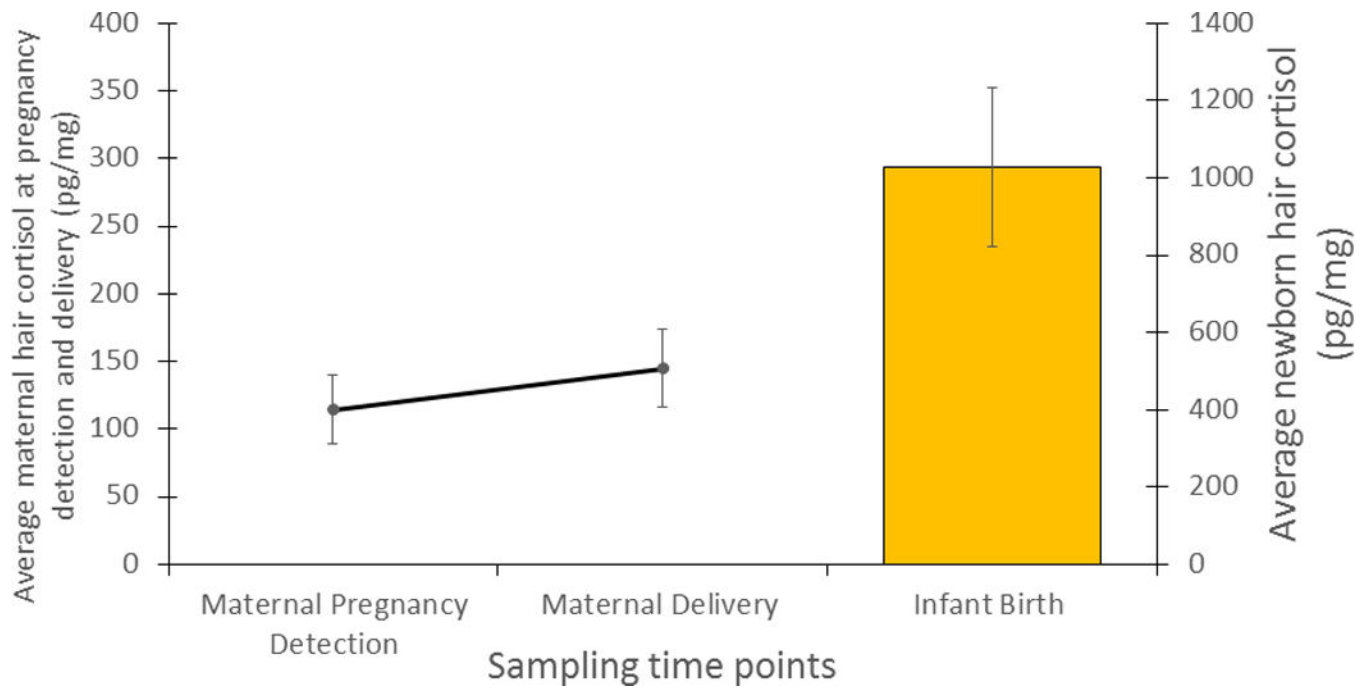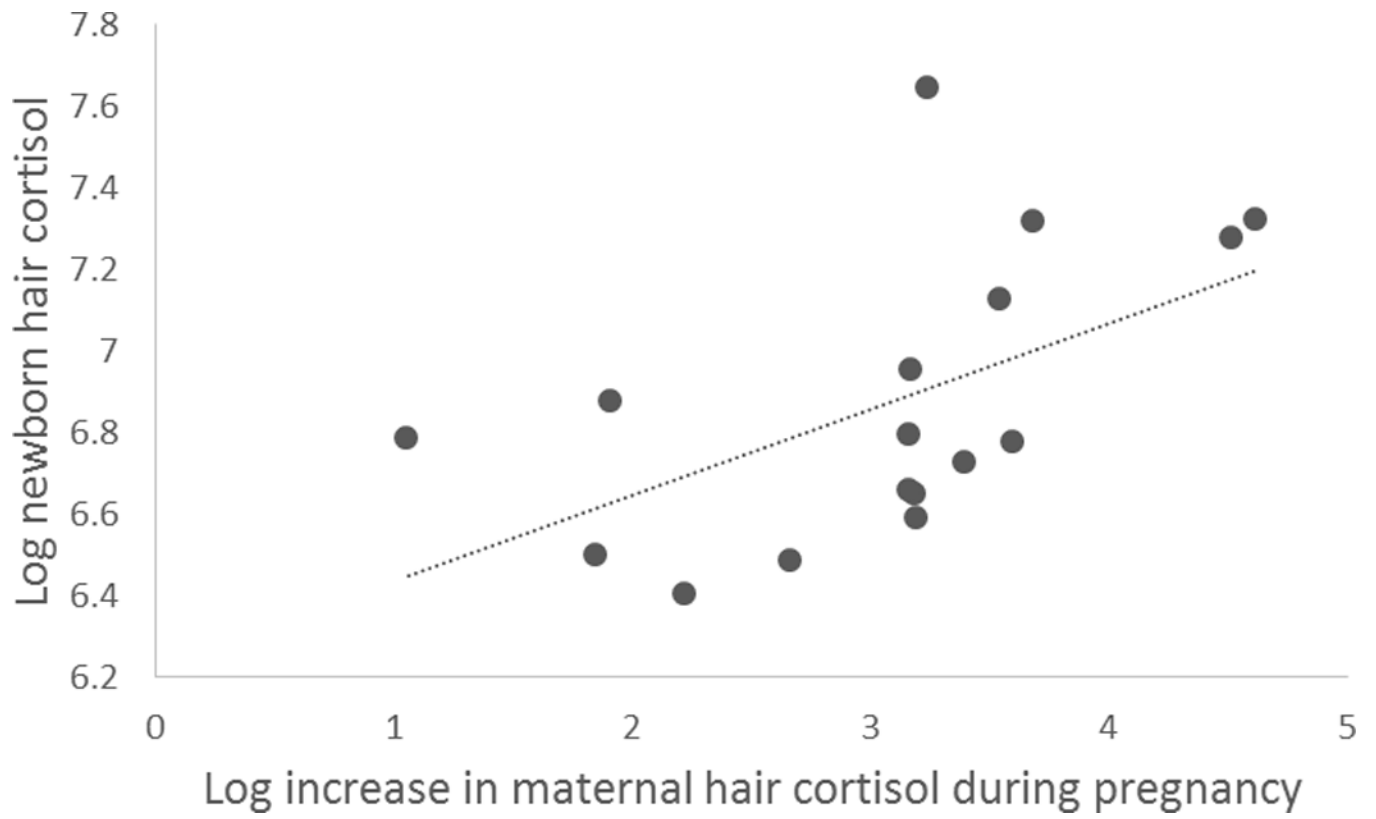
Figure 1:

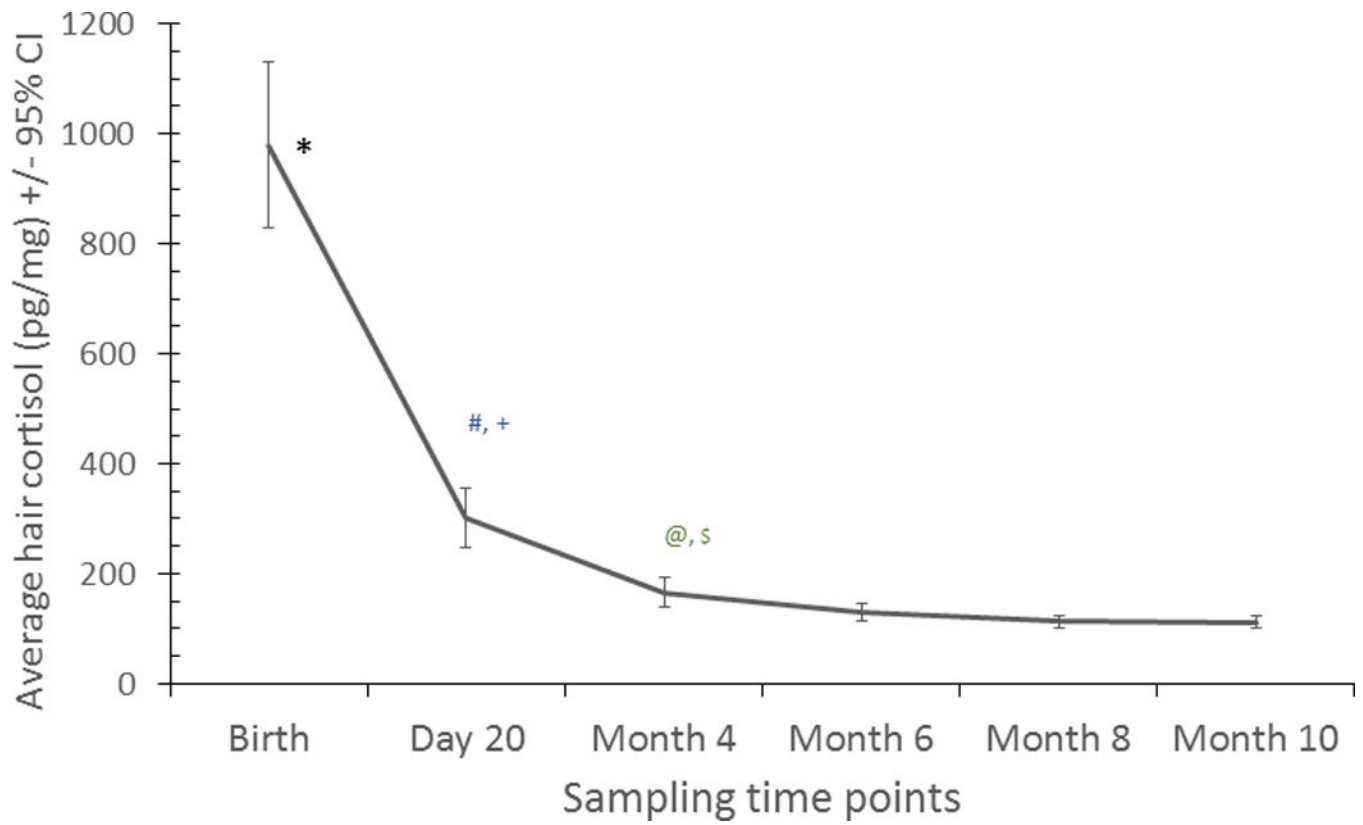Average hair cortisol concentrations (pg/mg) for maternal and newborn babies (N=17), Errors bars are 95% confidence intervals.
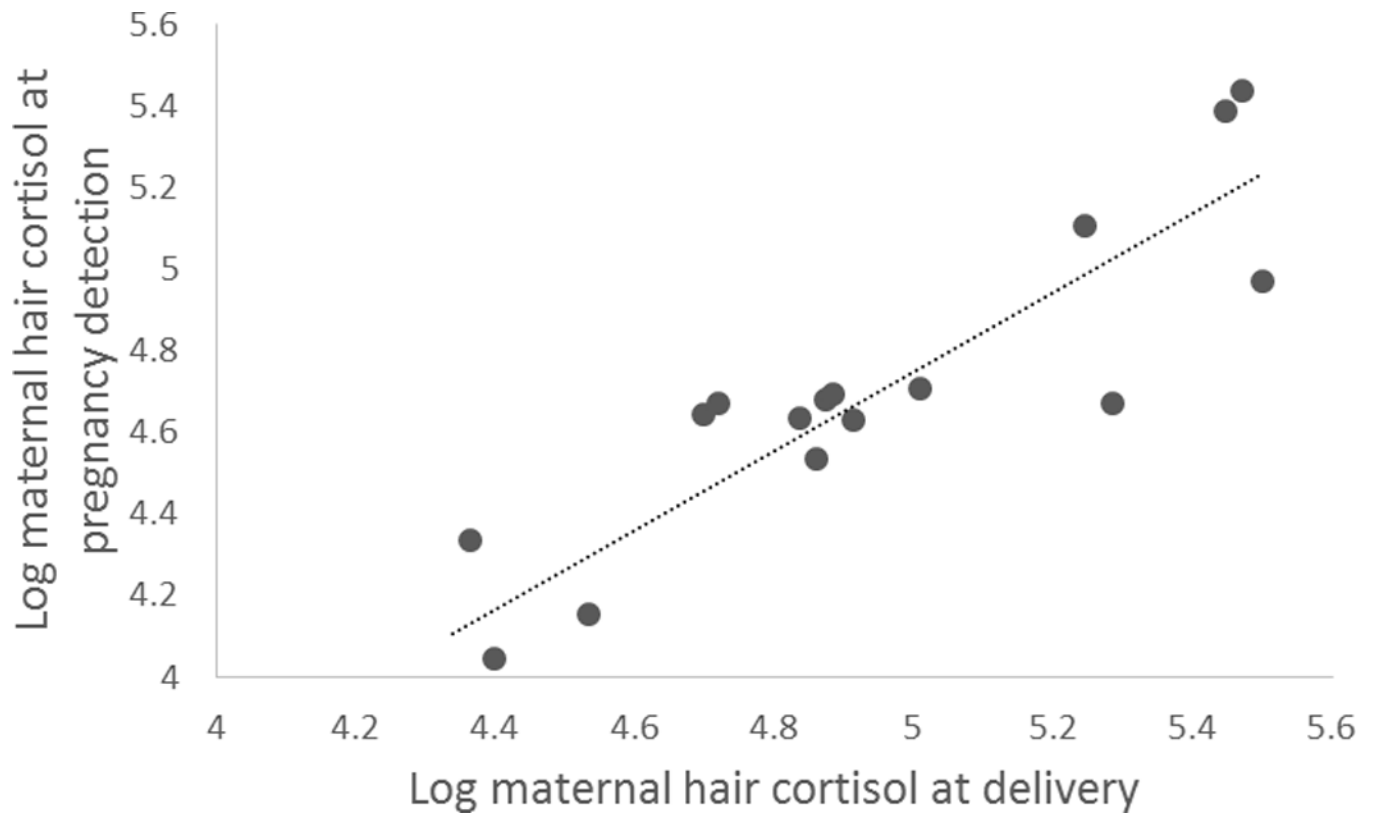
Figure 2:
Scatterplot showing relationship between long maternal hair cortisol concentration at pregnancy detection and delivery (r=.90, p<.001).

Figure 3:
Scatterplot demonstrating the relationship between the rise in maternal hair cortisol
concentrations over pregnancy and levels of newborn cortisol (r=0. 55, p=.02).

Figure 4. Average (95% Cl) hair cortisol concentrations , Birth to 10 Months of Age.
Results of tests: *Birth vs Day 20, Months 4,6,8,10 (p<0.001), # Day 20 vs Month 4
(p<0.005), + Day 20 vs Months 6, 8, 10 (p<0.001), @ Month vs Month 8 (p<0.05), $ Month
4 vs Month 10 (<0.005).
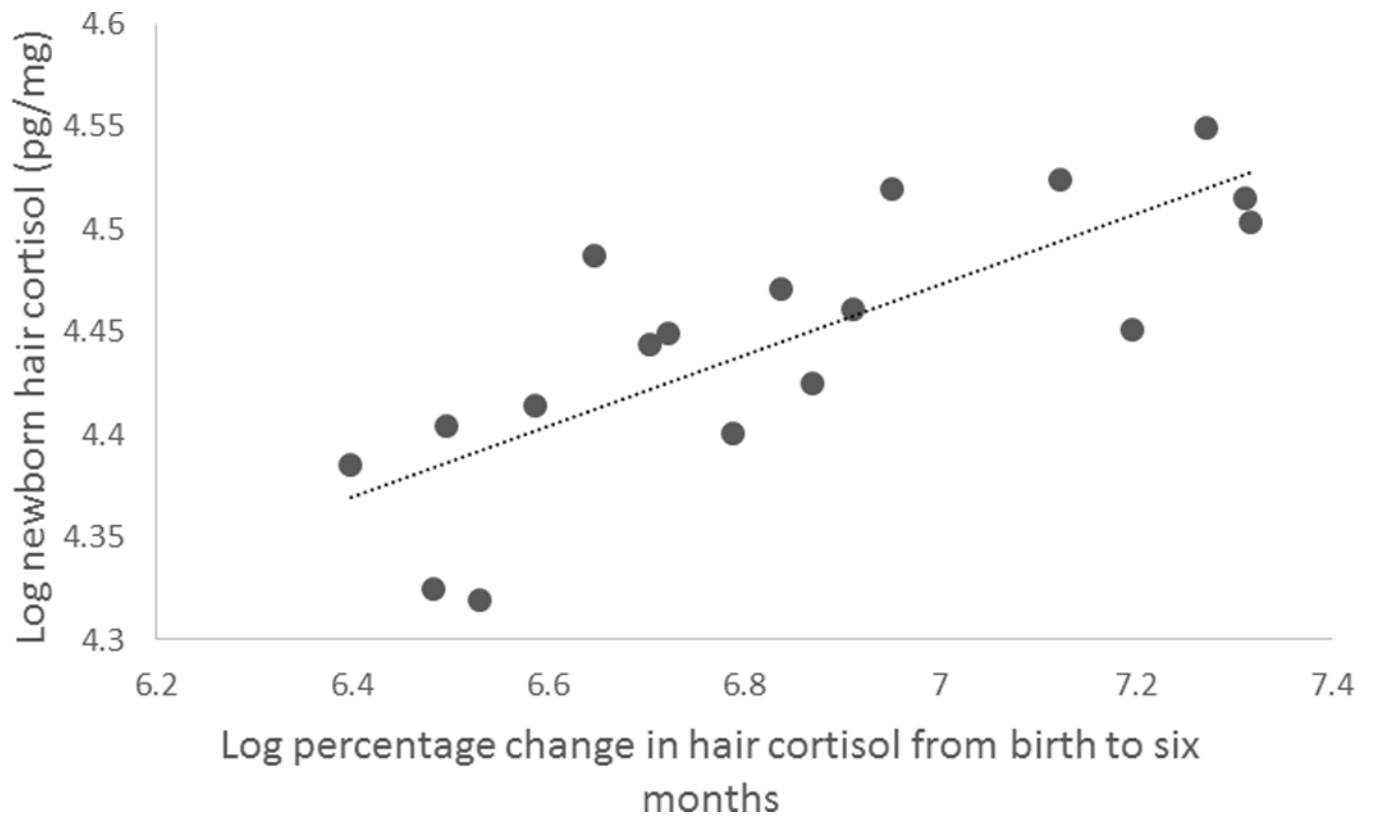
Figure 5:
Scatterplot illustrating the relationship between newborn hair cortisol concentrations and change in hair cortisol from birth to 6 months of age (r=.79, p<.001).

Table 1:

List of subject characteristics and hair cortisol concentrations for maternal-newborn (N=17) and infancy (N=18) analyses.

Mother-Infant Dyad	Primiparous or Multiparous	BirthDate	Delivery Type	Experimental Intervention[1]	Infant Gestation Age at Birth	Infant Sex	Birthweight	Hair Cortisol Concentrations (pg/mg)									
								Maternal Pregnancy Detection	Maternal Delivery	Maternal Difference[2]	Maternal % Difference[3]	Infant Birth	20 Days	4 Months	6 Months	8 Months	10 Months
Mother-Infant Dyads in Maternal-Newborn and Infancy Analyses																	
1	Multiparous	07/05/13	C-section	No	178	Male	660	229.0	238.3	9.3	4.1%	601.4	319.4	194.6	119.4	89.4	81.3
2	Primiparous	07/17/13	C-section	Yes	173	Female	535	218.1	232.6	14.5	6.6%	655.0	423.6	253.8	160.8	100.7	97.1
3	Multiparous	07/20/13	C-section	No	179	Male	495	52.2	76.6	24.4	46.7%	771.5	446.0	74.1	86.8	67.3	52.8
4	Multiparous	07/22/13	C-section	Yes	180	Female	559	103.2	110.0	6.8	6.6%	964.8	133.3	123.4	160.0	113.8	118.5
5	Multiparous	08/10/13	Natural	No	168	Female	480	143.3	245.1	101.8	71.0%	1509.2	293.8	151.6	147.2	121.9	129.7
6	Primiparous	09/03/13	C-section	No	179	Male	600	106.3	198.0	91.7	86.3%	1441.9	512.3	146.4	79.4	129.0	116.9
7	Multiparous	09/17/13	Natural	No	180	Female	500	56.9	81.5	24.6	43.2%	727.4	149.5	185.6	127.4	106.2	94.7
8	Primiparous	10/02/13	Natural	Yes	174	Male	535	63.2	93.2	30.0	47.5%	833.3	179.5	182.3	121.0	114.4	117.0
9	Primiparous	10/20/13	Natural	No	173	Female	485	101.8	136.5	34.7	34.1%	1241.6	453.2	298.8	97.9	86.3	139.9
10	Primiparous	12/08/13	Natural	Yes	173	Male	480	107.1	131.1	24.0	22.4%	1046.4	220.5	157.1	87.3	92.8	122.8
11	Multiparous	06/15/14	Natural	No	178	Male	640	102.6	126.3	23.7	23.1%	890.0	227.5	149.7	165.7	119.2	105.7
12	Primiparous	08/01/14	Natural	No	180	Female	490	106.1	112.5	6.4	6.0%	663.6	271.3	122.9	121.4	133.1	116.4
13	Primiparous	09/09/14	Natural	Yes	179	Female	545	110.1	150.1	40.0	36.3%	1500.0	343.3	141.0	131.2	88.8	76.3
Mother-Infant Dyads in Maternal-Newborn Analysis Alone																	
14	Primiparous	08/06/13	Natural	No	163	Female	380	92.6	129.2	36.6	39.5%						
15	Multiparous	01/04/15	Natural	No	176	Female	540	164.3	190.0	25.7	15.6%						
16	Multiparous	07/18/15	C-section	Yes	185	Female	520	108.7	132.4	23.7	21.8%						
17	Primiparous	07/14/15	Natural	No	175	Female	605	75.9	78.8	2.9	3.8%						
Mother-Infant Dyads in Infancy Analysis Alone																	
14	Multiparous	06/01/13	Natural	No	171	Female	490					1335.4	378.0	163.8	192.1	148.9	138.9
15	Primiparous	06/13/13	C-section	No	179	Female	470					817.6	324.5	186.1	122.7	130.9	120.5
16	Primiparous	08/30/13	C-section	No	178	Female	500					686.8	250.7	215.7	171.5	133.7	124.0
17	Primiparous	04/16/14	Natural	No	172	Female	595					934.0	233.2	103.9	118.6	103.6	118.6
18	Multiparous	06/03/14	Natural	No	175	Female	515					1005.6	250.7	135.2	136.3	150.0	131.1

[1] Experimental Intervention = Females were tested for wearing a jacket for tethering study during pregnancy

[2] Maternal Difference Hair Cortisol Concentration = Maternal Delivery Hair Cortisol Concentration - Maternal Pregnancy Detection Hair Cortisol Concentration

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[3] Maternal % Difference Hair Cortisol Concentration = Maternal Difference Hair Cortisol Concentration / Maternal Delivery Hair Cortisol Concentration

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Table 2:

Pearson (r) correlations for association between newborn hair cortisol (HC) concentrations (N=18) and decreases in hair cortisol during first 10 months of life

	20 Day HC	4 Month HC	6 Month HC	8 Month HC	10 Month HC
Birth HC Pearson Correlation	0.52 *	0.70 ****	0.79 ****	0.78 ****	0.73 ****
20 Day HC Pearson Correlation		0.53 *	0.36	0.26	0.16
4 Month HC Pearson Correlation			0.74 ****	0.59 **	0.67 ***
6 Month HC Pearson Correlation				0.83 ****	0.75 ***
8 Month HC Pearson Correlation					0.90 ****

*
=p .05

**
=p .01

***
= p .005

****
=p .001