FACTORS AFFECTING FECAL GLUCOCORTICOID LEVELS IN SEMI-FREE-RANGING FEMALE MANDRILLS (*MANDRILLUS SPHINX*)

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ABSTRACT

Subordinate female cercopithecine primates often experience decreased reproductive success in comparison to high-ranking females, with a later age at sexual maturity and first reproduction, and/or longer interbirth intervals. One explanation that has traditionally been advanced to explain this is high levels of chronic social stress, resulting from agonistic and aggressive interactions in subordinates and leading to higher basal levels of glucocorticoids. We assessed the relationships among fecal cortisol levels and reproductive condition, dominance rank, degree of social support and fertility in female mandrills (*Mandrillus sphinx*) living in a semi-free-ranging colony in Franceville, Gabon. Lower-ranking females in this colony have a reproductive disadvantage relative to higher-ranking females, and we were interested in determining whether this relationship between dominance rank and reproductive success is mediated via stress hormones. We analyzed 340 fecal samples from 19 females, collected over a 14 month period. We found that pregnant females experienced higher fecal cortisol levels than cycling or lactating females. This is similar to results for other primate species, and is likely due to increased metabolic demands and interactions between the hypothalamus-pituitary-adrenal axis, estrogen and placental production of corticotrophin releasing hormones during pregnancy. There was no influence of dominance rank on fecal cortisol levels, suggesting that subordinate females do not suffer chronic stress. This may be because female mandrills have a stable social hierarchy, with low levels of aggression and high social support. However, we found no relationship between matriline size, as a measure of social support, and fecal cortisol levels. Subordinates may be able to avoid aggression from dominants in the large enclosure, or may react only transiently to specific aggressive events, rather than continuously expecting them. Finally, we found no relationship between fecal cortisol levels and fertility. There was no difference in fecal cortisol levels between conceptive and non-conceptive cycles, and no significant relationship between fecal cortisol level and either the length of post-partum amenorrhea or the number of cycles before conception. This suggests that the influence of dominance rank on female reproductive success in this population is not mediated via chronic stress in subordinate females, and that alternative explanations of the relationship between social rank and reproduction should be sought.

KEYWORDS

stress; fecal cortisol; dominance rank; reproduction; allostatic load

INTRODUCTION

Among cooperative breeders, reproduction is monopolised by dominant group members, while subordinates are often completely reproductive suppressed [Emlen 1991; Solomon and French 1997]. Among non-cooperatively breeding group-living species, subordinate individuals also often suffer reduced reproductive success. For example, subordinate female cercopithecine primates may have a later age at sexual maturity and first reproduction (leading to a shorter reproductive lifespan), and/or longer interbirth intervals (decreased rate of offspring production) [Altmann et al. 1988; Bercovitch and Strum 1993; Bulger and Hamilton 1987; Harcourt 1987; Setchell et al. 2002; Smuts and Nicolson 1989; van Noordwijk and van Schaik 1999; Wasser et al. 1998]. Reduced reproductive success in subordinate females may be due to a number of factors, including reduced access to resources [e.g. Pusey et al. 1997], harassment by dominants [Wasser and Barash 1983], and/or a lack of access to males [e.g. Zinner et al. 1994]. In addition, high levels of chronic social stress, resulting from agonistic and aggressive interactions that lead to higher basal levels of glucocorticoids (GCs) have traditionally been thought to play a major role in reducing reproductive success in subordinates [Dunbar 1988; von Holst 1998; Wasser and Barash 1983]. Activation of the hypothalamus-pituitary-adrenal (HPA) axis during the endocrine stress response results in the release of these ‘stress hormones’, which mobilize energy by stimulating the release of glucose into the bloodstream and lipolysis in adipose tissue, and enhance delivery of glucose, fatty acids and triglycerides to skeletal muscle and the brain [Sapolsky 1994; Wingfield et al. 1998]. GCs also divert energy from various costly physiological processes that are not required for immediate survival, including digestion, energy storage, growth, immunity and reproduction [reviews in Sapolsky 2000; Sapolsky 2002]. Thus, while adaptive in the short-term, chronic GC elevation has serious negative effects on the organism, including reproductive failure and decreased resistance to disease [Sapolsky 2002].

Studies of the relationships between rank and GC levels in female primates have yielded mixed results. For example, while some studies of female primates have found that subordinate individuals exhibit high levels of GCs [e.g. long-tailed macaques, *Macaca fascicularis*, Shively et al. 1997], others have found the reverse, that dominant animals have high GCs [e.g. common marmosets, *Callithrix jacchus*, Abbott et al. 1998; ring-tailed lemurs, *Lemur catta*, Cavigelli 1999; Cavigelli 2003; cotton top tamarins, *Saguinus oedipus*, Ziegler et al. 1995], and still others have found no consistent relationship between social rank and GCs [e.g. chacma baboons, *Papio hamadryas ursinus*, Engh et al. 2006b; talapoins, *Miopithecus talapoin*, Keverne et al. 1984; black tufted-ear marmosets, *Callithrix kuhli*, Smith and French 1997; long-tailed macaques, Stavisky et al. 2001; van Schaik et al. 1991]. Meta-analyses of primates [Abbott et al. 2003] and group-living animals in general [Goymann and Wingfield 2004] have concluded that the physiological costs associated with different social ranks are determined by the way in which rank is achieved and maintained, rather than by the rank position itself. Thus the magnitude and direction of the relationship between GCs and rank is thought to be influenced by rank stability, whether high rank is fought for or follows a convention (such as inheritance or queuing), the degree to which dominants harass subordinates, and the level of social support available [Abbott et al. 2003; Goymann and Wingfield 2004]. Goymann and Wingfield [2004] have formalized this relationship between social status and stress hormones in the “allostatic load” model. “Allostatic load” refers to the cumulative physiological costs of maintaining homeostasis in the face of both predictable and unpredictable events [McEwen and Wingfield 2003]. An increase in allostatic load is typically accompanied by a rise in GC levels, and the relative allostatic load of social status predicts whether dominants or subordinates express higher or lower GC concentrations [Goymann and Wingfield 2004].

In addition to possible influences of social status, aggression and social support, GC levels in female primates are influenced by reproductive condition, male immigration, infanticide risk, rank instability, predation and seasonal changes in resource availability. For example, stage of the reproductive cycle influences GC levels in chacma baboons [Engh et al. 2006b; Weingrill et al. 2004], ring-tailed lemurs [Cavigelli 1999], cotton-top tamarins [Ziegler et al. 1995], golden lion tamarins [*Leontopithecus rosalia*, Bales et al. 2005] and common marmosets [Saltzman et al. 1994]. This is likely due to the increased metabolic demands of pregnancy and lactation, and to interactions between the hypothalamus-pituitary-adrenal axis, estrogen and placental production of corticotrophin releasing hormones during pregnancy. Fecal corticosterone levels in lactating female chacma baboons increased in response to the arrival of potentially infanticidal immigrant males, and increased even further when infanticide occurred [Engh et al. 2006b]. In contrast, fecal corticosterone levels of cycling and pregnant females, who were not vulnerable to infanticide, did not change [Engh et al. 2006b]. Fecal corticosterone levels were also elevated in response to rank instability among females, particularly if the female's own rank was at risk of decreasing [Engh et al. 2006b], and in response to the death of a close relative [Engh et al. 2006a]. Finally, harsh climatic conditions and low food availability are associated with high fecal cortisol levels in ring-tailed lemurs [Cavigelli 1999; Pride 2006] and chacma baboons [Weingrill et al. 2004], two species that experience high seasonal stress.

The use of fecal samples to examine behavior-endocrine interactions has become increasingly popular in recent years [Hodges and Heistermann 2003; Touma and Palme 2005; Whitten et al. 1998]. Unlike serum collection, fecal sampling does not require handling, restraint or anesthesia, all of which can provoke stress responses that will influence GC levels [Whitten et al. 1998]. Fecal samples can thus be collected from wild or semi-free-ranging animals more regularly than serum samples. Further, fecal GC levels represent the cumulative secretion and elimination of hormones over a period of hours, providing a more integrated measure of adrenocortical activity than point serum samples, and diminishing the influence of the pulsatile and episodic patterns of GC secretion [Whitten et al. 1998]. In this study we investigated the relationships between variation in fecal cortisol levels and age, reproductive condition, dominance rank, degree of social support and fertility in female mandrills (*Mandrillus sphinx*, Cercopithecinae) living in a semi-free-ranging colony at the Centre International de Recherches Médicales, Franceville (CIRMF), Gabon. Female mandrills in this colony live in stable, inherited dominance relationships, and lower-ranking females have a reproductive disadvantage relative to higher-ranking females, experiencing their first sexual cycles on average six months later, giving birth for the first time at a later age, and undergoing longer inter-birth intervals [Setchell et al. 2002; Setchell and Wickings 2004]. We were interested in determining whether this relationship between dominance rank and reproductive success is mediated via stress hormones [e.g. Dunbar 1988; Wasser and Barash 1983]. We also examined the influence of alpha male turn-over and male rank instability on fecal cortisol levels in females.

We predicted that reproductive condition should influence fecal cortisol levels in female mandrills, based on results for other cercopithecine species [Beehner et al. 2005; Engh et al. 2006b; Weingrill et al. 2004]. Specifically, pregnant females should experience higher fecal cortisol levels than either cycling or lactating females, and fecal cortisol levels should increase as pregnancy progresses and circulating levels of ovarian hormones increase. We derived our predictions for the relationship between fecal cortisol and dominance rank from two alternative hypotheses. First, we estimated the relative allostatic load of dominance and subordinance in female mandrills, following criteria in Goymann and Wingfield [2004]. The female dominance hierarchy in mandrills is stable over years, with daughters ranking immediately below their mother in reverse birth order, as is typical for female cercopithecine primates [Setchell 1999]. The allostatic load of acquiring dominance is thus low (allostatic load score 1), while the costs of maintenance are moderate (allostatic load score 2), giving a total score of 3. For subordinates, the degree of threat from dominants is moderate (they are regularly threatened with low level aggression, score 2), and some coping mechanisms are available, including the ability to escape from conflict in a very large enclosure, and kin support (score 1). The total score for dominant and subordinate female mandrills is equal (3). The allostatic load model thus predicts no overall relative cost to subordinance in female mandrills, and no difference in fecal cortisol levels between dominant and subordinate females. These scores are the same as those assigned to long-tailed macaques by Goymann and Winfield [2004]. Alternatively, if dominant females employ randomly timed aggression on randomly selected targets to create continuing uncertainty in subordinates about when and if they will be attacked, as proposed by Silk [2002], then low-ranking animals should suffer from chronic, low-level stress, despite the stable dominance hierarchy. Under this model, we predicted higher fecal cortisol levels in subordinate female mandrills relative to dominants. Finally, if kin support is an important social buffer against stress [as shown by Abbott et al. 2003], then we predicted that females living in larger matrilines (with more support available) would have lower fecal cortisol levels.

If the relationship between dominance rank and reproductive success in the mandrill colony is mediated via stress hormones [e.g. Dunbar 1988; Wasser and Barash 1983], then high fecal cortisol levels should be linked to reduced fertility in females. If this is the case, then we predicted that fecal cortisol levels would be (i) higher in non-conceptive cycles than in conceptive cycles, (ii) higher in females that experienced longer post-partum amenorrhea than in females that resumed cycling more quickly, and (iii) higher in females that required more cycles to conceive than those that conceived easily.

Lastly, we investigated the influence of male rank stability on fecal cortisol levels in females. During the course of the study, the alpha male died and was replaced by another male, who was subsequently replaced by a second new alpha male. Both new alpha males were natal males, and were likely to have mated during the previous mating season, meaning that there was little risk of infanticide to lactating females. Moreover, the male take-overs occurred at a time when few females were lactating, and those that were were nearing the end of their post-partum amenorrhea. Based on recent findings in wild chacma baboons [Engh et al. 2006b], showing that fecal corticosterone levels of females who were not vulnerable to infanticide were not affected by changes in male ranks, we expected that these changes in male rank would have little influence on female GC levels.

METHODS

Study population and subjects

Mandrills inhabit the dense rain-forest of Gabon, Equatorial Guinea, southern Congo, and southern Cameroon, and it has proved impossible to habituate and follow known individuals in the wild [Abernethy et al. 2002; Harrison 1988]. However, the semi-free-ranging colony of mandrills at CIRMF, Gabon, represents a useful compromise between captivity and the wild condition. The colony was established in 1983/4, when 15 animals (seven males, eight females) were released into a 6.5 ha forest enclosure (E1). There were no subsequent additions to the colony, other than by breeding, although animals were occasionally been removed, and in 1994 a second semi-free-ranging group was established in a smaller enclosure (E2, 3.5 ha) by transferring 17 mandrills (including 6 adult females and 4 adult males) from the first enclosure. The animals foraged freely and received daily supplements of monkey chow, fruit and vegetables in a designated feeding area with a concrete floor that is hosed down before provisioning. Water was always available from a stream, which runs through both enclosures. Breeding in the colony is moderately seasonal, with 63% of peri-ovulatory periods occurring between July and September, and only 6% between December and April [Setchell and Wickings 2004], and a corresponding birth peak in January to March [Setchell et al. 2002]. Mean gestation length is 5.8 months [175 days, Setchell et al. 2002], with a mean duration of post-partum amenorrhea following the birth of a live infant of 7 months [208 months, Setchell and Wickings 2004].

This study concentrated on E1, and was conducted between February 2004 and March 2005. In February 2004 there were 75 animals living in E1 (43 females, 32 males). Nineteen infants were born into the group during the study, one infant and one adult male died, and one adolescent male escaped. The size and age-sex composition of the study group during the study period correspond to the smaller end of group sizes observed in the wild [Rogers et al. 1996]. Subjects were all 19 females in E1 that had already produced at least one infant at the beginning of the study. Subjects had a mean age at the beginning of the study of 11.0 yr (range 4.7-25.1 yr), a mean parity of 6.3 (range 1-18) and were members of 5 different matrilines (i.e. descendants of five different founder females). Female mandrills do not attain their full adult body mass and length until 7 yr, [Setchell et al. 2001]. Longevity is unknown, as no female in the colony has yet died of old age. However, females aged 20+ yr appear physically elderly, and two of the four females aged 20+ yr have ceased to conceive. Subjects were thus divided into young (<7yr at the beginning of the study, n=7), mid (10-20 yr, n=10) and old (>20 yr, n=4) age groups.

The reproductive state of females was noted daily as cycling (females in any stage of the menstrual cycle, during which females show conspicuous perineal swellings [Dixson 1998]), pregnant (assigned post hoc from the birth of an infant, beginning with the final detumescence of the perineal skin), lactating (the period following the birth of an infant to the resumption of cycling) or other (not pregnant, lactating or cycling).

Female behavior was observed *ad libitum* during twice daily observation periods (approx. 10h00-11h30 and 15h30-17h30). Dominance rank was calculated using dyadic interaction matrices, including all interactions where one female avoided or fled when another female approached. These dominance ranks were identical to those previously recorded for the females [e.g. Setchell et al. 2002;

Setchell and Wickings 2004], with the only changes being those expected due to births and deaths, confirming that the females lived in stable, inherited dominance relationships. Females were classified as high- (upper-quartile), mid- (inter-quartile range) or low- (lower quartile) ranking, depending upon the proportion of females that they dominated [Setchell et al. 2001].

Fecal sampling

A total of 348 fecal samples were collected from the study females between February 2004 and March 2005 (no samples were collected in March or December 2004). Samples were collected when females defecated in the feeding area during either morning (10h00 – 11h30) or afternoon (15h30 – 17h30) observation periods. The mandrills were very well habituated to human presence at the feeding area and samples were collected immediately after defecation. The identity of the individual, date, time (am or pm) and consistency of the sample were noted. Feces were homogenized using a stick, and a portion (mean±SEM = 6.6±0.1 g) was placed in a collection tube with 40 ml of 90 % ethanol immediately and stored until extraction. Only 8 samples were noted as being runny (diarrheic); these were not used in analyses. We attempted to collect three samples per month for each female, with a mean of 17.6±10.3 samples per female (range 3-36).

Extraction

Fecal samples were homogenized in their storage ethanol, vortexed for 1 minute, and shaken for three hours. They were then centrifuged for 15 minutes at 2000 rpm. A 2 ml aliquot of the supernatant was dried under air at 40 ^{0}C, and then re-suspended in 1 ml EIA PBS (0.1 M phosphate buffered saline, pH 7.0, with 0.1% bovine serum albumin). The fecal pellet was dried to constant mass at 60 ^{0}C to determine the dry mass of the sample. Extraction efficiency was determined by measuring recovery of ^{3}H–cortisol (3010 counts per minute) added to ten samples prior to extraction. Mean extraction efficiency was 84.4±1.4 %. Hormone concentrations were corrected for this extraction loss and expressed as ng of hormone per mg of dry feces. Mean dry fecal mass was 1.65±0.03 g (range 0.39-3.69 g).

Cortisol assays

Fecal extracts were measured for immunoreactive cortisol by microtitreplate enzyme immunoassay (EIA), using an antiserum (R4866) raised against a steroid bovine albumin in rabbit [Munro and Stabenfeldt 1985], and horseradish peroxide as a label. This antibody has cross reactivities of 96% with prenisolone, 66% with prednisone, 60% with cortisone, 2.5% with corticosterone and < 1% with various other steroids [Ziegler et al. 1995].

We validated the assay immunologically by demonstrating parallelism, accuracy, and sensitivity. Serial dilutions of pooled fecal extracts gave displacement curves parallel to that obtained for the cortisol standard for 30-90 % binding, indicating that the amount of cortisol measured varied directly with the volume of extract for this portion of the curve. Accuracy for fecal extracts added to the standard curve was a mean of 100.9±6.8 % ($r^2 = 0.993$, n=12). Assay sensitivity was 1.95 pg/well.

For practical and ethical reasons it was not possible to conduct an ACTH challenge [e.g. Wasser et al. 2000] to establish whether fecal assays accurately reflect acute adrenal activation, and thus to physiologically validate the cortisol antibody for the CIRMF mandrills. We therefore took advantage of opportunistic events to test whether fecal cortisol levels reflected stressful states or experiences. We did this in two ways. First, we compared cortisol measures in fecal samples from 16 male mandrills that lived in two separate groups, but were then combined in one group (a presumed stressor). Levels of immunoreactive cortisol were significantly higher following mixing of the two groups (paired $t_{15} = 2.29$, p=0.037). This pattern suggest that the antibody can track fluctuations in metabolites that provide biologically relevant information regarding adrenal status, and that elevations in immunoreactive cortisol in the fecal samples may reasonably be regarded as indicative of the physiological stress response in these mandrills. Second, we compared fecal cortisol levels for female mandrills on the day following a group capture with those obtained one or two days prior to capture (a stressful experience for both captured and non-captured animals). We obtained paired for 13 females, and found that fecal cortisol levels were significantly higher following a capture than previously (paired $t_{12} = 2.29$, p = 0.041).

Fecal extracts were diluted 1:6 in assay buffer (0.1 M phosphate buffered saline, pH 7.0, with 0.1% bovine serum albumin) and 50 µl aliquots assayed along with 50 µl aliquots of reference standard in doubling dilutions (range 1.95-1000 pg/well). Samples were re-run if duplicates had coefficients of variation greater than 5%. Samples binding >90% or <30% were re-diluted and re-assayed. The intra-assay coefficient of variation for a subset of 30 samples was 3.4±1.9 %. High (HQC) and low (LQC) quality controls, consisting of a pool of all female samples, were run in duplicate on each plate. Inter-assay variation for these controls was high (HQC 35.3%, LQC 34.3%, n=24), indicating assay drift. However, HQC and LQC concentrations were correlated highly across all plates (r>0.9, n=24), and values for the same samples on different plates also yielded highly correlated results (r>0.9, n=33). We therefore normalized all data to the mean of the quality controls on the same plate as follows: we calculated the mean of the high and low QCs for each plate, and took the mean of these values across all plates. We then adjusted the assay results for each plate by the difference between the mean for the individual plate and the overall mean for all plates. This reduced inter-assay variation to 8.0% (HQC) and 2.8% (LQC). We also ensured that there was no confound between the assay plate number and any of our predictor variables.

Statistical analysis

Fecal cortisol levels were normalized via log transformation. We used a general linear mixed model (GLMM) to assess the effects of categorical variables on fecal cortisol levels. GLMMs extend general linear models by the inclusion of random effects in the predictor. We entered all fecal cortisol measurements into the analysis, and included individual identity as a random factor in the model because we sampled the same individuals repeatedly, using variance components for the covariance structure.

Categorical predictor variables were as follows:

1. Age : young, mid and old (see above)
2. Dominance rank (see above)

3. Matriline size, as a measure of social support: Defined as the number of reproductive females in a female's matriline, and divided into small (1-6 females, n=13 females) vs. large (11 females, n=6 females)
4. Reproductive condition (cycling, pregnant, lactating, see above). Fecal samples were matched with the reproductive condition of a female on the previous day, to allow for the time lag to peak steroid excretion [Bahr et al. 2000]. Extending this time lag to 2 days [Shideler et al. 1993] did not alter our the significance of our results. Insufficient data were available to examine fecal cortisol levels in females that were not pregnant, lactating or cycling, and these data were not included in analyses.

Hormone concentrations change over time when fecal samples are stored in ethanol, although these patterns are dampened by storage at sub-zero temperatures [Khan et al. 2002]. We therefore entered the number of months for which the samples were stored prior to extraction as a covariate in analyses to detect any effects related to degradation of hormones over time. We also included a categorical variable to detect any effect of circadian rhythm on fecal cortisol levels (am vs. pm samples). We included main effects, and the interaction term dominance rank*reproductive condition (to determine whether there were any rank specific influences of reproductive condition on fecal cortisol levels) in the model.

We used additional GLMMs with identity as a random factor to account for multiple measurements on individual females to explore the effects of reproductive condition on fecal cortisol levels as follows. First, we used pairwise analyses of different reproductive states (cycling vs. pregnant, pregnant vs. lactating, cycling vs. lactating). Second, we divided gestation and lactation into 6 and 7 monthly intervals, respectively, with the day of parturition termed day 0, to examine changes in fecal cortisol across the reproductive cycle in more detail.

We investigated the relationship between fecal cortisol levels and female fertility in three ways. First, we compared fecal cortisol levels in conceptive vs. non-conceptive cycles (categorical variable). We used all samples available for females in their follicular phase (determined from sexual swelling records), as insufficient samples were available to compare only peri-ovulatory periods of conceptive and non-conceptive cycles. Second, we compared fecal cortisol levels in lactating females with the time to resumption of cycling (as a covariate). Third, we compared fecal cortisol in cycling females with the number of cycles required to conceive (categorical variable).

Finally, to examine the influence of male rank stability on fecal cortisol levels in females we compared the stable period February-July 2004 with the period when two new males took over as alpha in swift succession (August-October 2004). Male rank stability (stable vs. unstable) was included as an additional categorical variable in a second GLMM analysis, because this used only a subset of the dataset.

We did not investigate the influence of seasonality on cortisol levels because the seasonal variation in reproduction in the colony [Setchell et al. 2002; Setchell and Wickings 2004], meant that the two potential influences were confounded. While the daily provisioning of the colony should compensate for any influence of seasonality in calorie intake, and the monkey chow does not vary across the year, the types of fruits and vegetables provided do vary, as do the resources available in the enclosures, so diet may still influence cortisol levels. Further seasonal cues may also be provided by changes in daylength (albeit small in equatorial Africa), temperature, humidity or rainfall.

All statistical tests were two-tailed, with the statistical threshold set at $P = 0.05$. All tests were conducted using SPSS 14.0 for Windows.

Ethical statement

This research was approved by the Comité Régional d’Ethique Ile de France Sud (the committee responsible for research on animals at CIRMF, Gabon) and adhered to the legal requirements of the country in which the research was conducted (Gabon).

RESULTS

Of the variables tested, only reproductive condition significantly predicted fecal cortisol levels in female mandrills (GLMM: $F_{2, 161.2} = 8.826$, $p<0.001$). Dominance rank ($F_{2, 12.6} = 0.617$, $p=0.555$), age ($F_{2, 9.5} = 0.601$, $p=0.568$), matriline size ($F_{1, 5.8} = 1.326$, $p=0.295$), length of time the fecal sample was stored ($F_{1, 277.4} = 0.153$, $p=0.696$) and whether a sample was collected during the am or pm observation sessions ($F_{1, 314.3} = 0.08$, $p = 0.930$) did not significantly predict fecal cortisol levels. The interaction between dominance rank and reproductive condition was also non-significant ($F_{4, 100.6} = 0.977$, $p=0.424$) suggesting that there was no rank-dependent influence of reproductive condition on fecal cortisol levels.

Subsequent analysis of reproductive stage and fecal cortisol measures showed that pregnant females had higher fecal cortisol levels than either cycling ($F_{1,42.1} = 11.698$, $p = 0.001$) or lactating females ($F_{1,168.9} = 12.151$, $p = 0.001$) (Fig. 1). There was no significant difference in fecal cortisol level between cycling and lactating females ($F_{1,217.0} = 0.620$, $p = 0.432$). Closer inspection of the data revealed that fecal cortisol rose from low levels in early pregnancy, and peaked in the second half of pregnancy (Fig. 2). Fecal cortisol decreased at parturition, and levels during lactation were higher than early pregnancy, but lower than late pregnancy.

We found no relationships between fecal cortisol levels and female fertility. There was no difference between fecal cortisol levels in the follicular phase of conceptive cycles and non-conceptive cycles ($F_{1,17.9} = 0.428$, $p = 0.522$). There was no relationship between fecal cortisol levels and either (i) the length of post-partum amenorrhea in lactating females ($F_{1, 139} = 0.469$, $p = 0.495$) or (ii) the number of cycles required to conceive in cycling females ($F_{4,74} = 1.215$, $p = 0.312$).

Finally, using a subset of the data to compare periods of stability and instability in male rank, we found that male rank stability did not influence fecal cortisol levels in females, based on a comparison of periods of stability and instability in male rank ($F_{2, 208} = 1.863$, $p = 0.158$).

DISCUSSION

Non-invasive methods are increasingly used in studies of non-human primates, and allow researchers to address questions and hypotheses that cannot otherwise be easily investigated [Hodges and Heistermann 2003; Touma and Palme 2005; Whitten et al. 1998]. In this study we demonstrated the feasibility and validity of fecal cortisol analyses for the assessment of HPA function in mandrills. Previous attempts to obtain useful measures of HPA activity using serum cortisol levels have been unsuccessful due to the effects of capture on the subject animals (EJW unpublished data). The methods reported here allowed will also be useful for studies of wild mandrills, as fecal samples can be collected even when habituation is impossible.

As predicted, reproductive condition had a significant effect on fecal cortisol concentrations in female mandrills, with the highest levels occurring during the second half of pregnancy. Elevated cortisol levels during late gestation have also been demonstrated in marmosets [*Callithrix kuhli*, Smith and French 1997], tamarins [Ziegler et al. 1995], ring-tailed lemurs [Cavigelli 1999], chacma baboons [Engh et al. 2006b; Weingrill et al. 2004] and humans [Lockwood et al. 1996]. These may be due to two possible causes: increased metabolic demands during pregnancy and stimulation of the HPA axis by ovarian hormones [McLean and Smith 1999]; and/or the elevation of circulating cortisol binding globulin during the latter stages of pregnancy [Demey-Ponsart et al. 2008]. Fecal cortisol levels in lactating females returned to the levels found in cycling females, as also found in other primate species [Cavigelli 1999; Engh et al. 2006b; Ziegler et al. 1995] [but see Weingrill et al. 2004].

We found no relationship between dominance rank and fecal cortisol levels in the semi-free-ranging female mandrills studied here. Subordinate females in this stable hierarchical situation did not appear to be subject to greater levels of chronic stress than high-ranking females. This is in accordance with findings for wild chacma baboons [Engh et al. 2006b; Weingrill et al. 2004], long-tailed macaques [van Schaik et al. 1991], and golden lion tamarins [Bales et al. 2005]. These findings support the predictions of the allostatic model of the relationship between social status and stress [Goymann and Wingfield 2004], rather than the alternative hypothesis [Silk 2002], which predicts that subordinates suffer chronic stress because dominants practice random acts of aggression on randomly selected individuals in order to maximize the negative impact on the victim. The observed lack of relationship between dominance and fecal cortisol levels may occur because subordinates are able to avoid dominants in the large enclosure, or because they react only transiently to specific aggressive events, rather than expecting them continuously [Beehner et al. 2005]. Subordinates may also experience social buffering via stress-reducing relationships [Abbott et al. 2003]. Although we found no influence of matriline size on fecal cortisol levels, it may be that all females had sufficient numbers of close allies to buffer social stress. Finally, contest over clumped provisioning does not appear to be sufficient to occasion chronic stress in subordinate females, perhaps because they use alternative foraging strategies. This is supported by the finding that dominant females are not necessarily larger or heavier than subordinates [Setchell 1999]. It is also possible that the costs of dominance are higher than traditionally assumed [e.g. Creel 2001], or that the provisioned nature of the colony buffers all females against the effects of stress.

We also found no relationship between female fertility and fecal cortisol levels. As in chacma baboons [Weingrill et al. 2004], there was no difference in fecal cortisol levels between conceptive and non-conceptive cycles and we found no significant relationship between fecal cortisol level and either the length of post-partum amenorrhea or the number of cycles before conception. The reproductive disadvantage experienced by subordinate females in the CIRMF mandrill colony [Setchell et al. 2002; Setchell and Wickings 2004] thus does not appear to be a consequence of chronic social stress compromising gonadal activity. A similar reproductive disadvantage in low-ranking females, but no relationship between excreted GCs and social status, has been reported for wild longtailed macaques [van Schaik et al. 1991]. Fertility suppression is also not associated with increased cortisol levels in marmosets and tamarins, where high GC levels are actually linked to increased fertility [Saltzman et al. 1994; Ziegler et al. 1995]. Taken together, these results suggest that the relationship between reproductive suppression and GCs is more complex than a simple model of low rank causing high stress and high GC levels, which in turn negatively impact reproductive function [Bercovitch and Ziegler 2002].

The question remains of why subordinate females in the mandrill colony begin reproduction later in life, require more cycles to conceive, and produce smaller offspring [Setchell et al. 2002; Setchell and Wickings 2004]. Other possible factors underlying the reduced reproductive success in subordinate females may include reduced access to resources or to mates, and/or reduced fertility. The first of these seems unlikely, as dominant females are neither larger, nor in better condition than subordinates [Setchell 1999]. It also seems unlikely that subordinate females require more cycles to conceive because they do not copulate sufficiently during earlier cycles to do so. Although top-ranking males prefer to mate with high-ranking females [Setchell and Wickings 2006], there are almost always more reproductive males available to mate than there are peri-ovulatory females [Setchell et al. 2005]. Finally, it may be that lower-ranking females are more likely to experience anovulatory cycles, for example, perhaps due to continued investment in previous offspring, and are less likely to conceive.

Lastly, our finding that male rank instability had no effect on female GC levels is not surprising, because although infanticide has been observed in the mandrill colony, the males that took over as alpha males posed little risk of infanticide to lactating females (see Introduction), and there were no very young infants present at the time of the take-overs. Thus, while the instability in the male dominance hierarchy might be expected to have an important influence on male GC levels [e.g. Bergman et al. 2005; Sapolsky 1992], females are only influenced by upheavals in the male hierarchy when these pose a direct risk to themselves or their offspring [Engh et al. 2006b].

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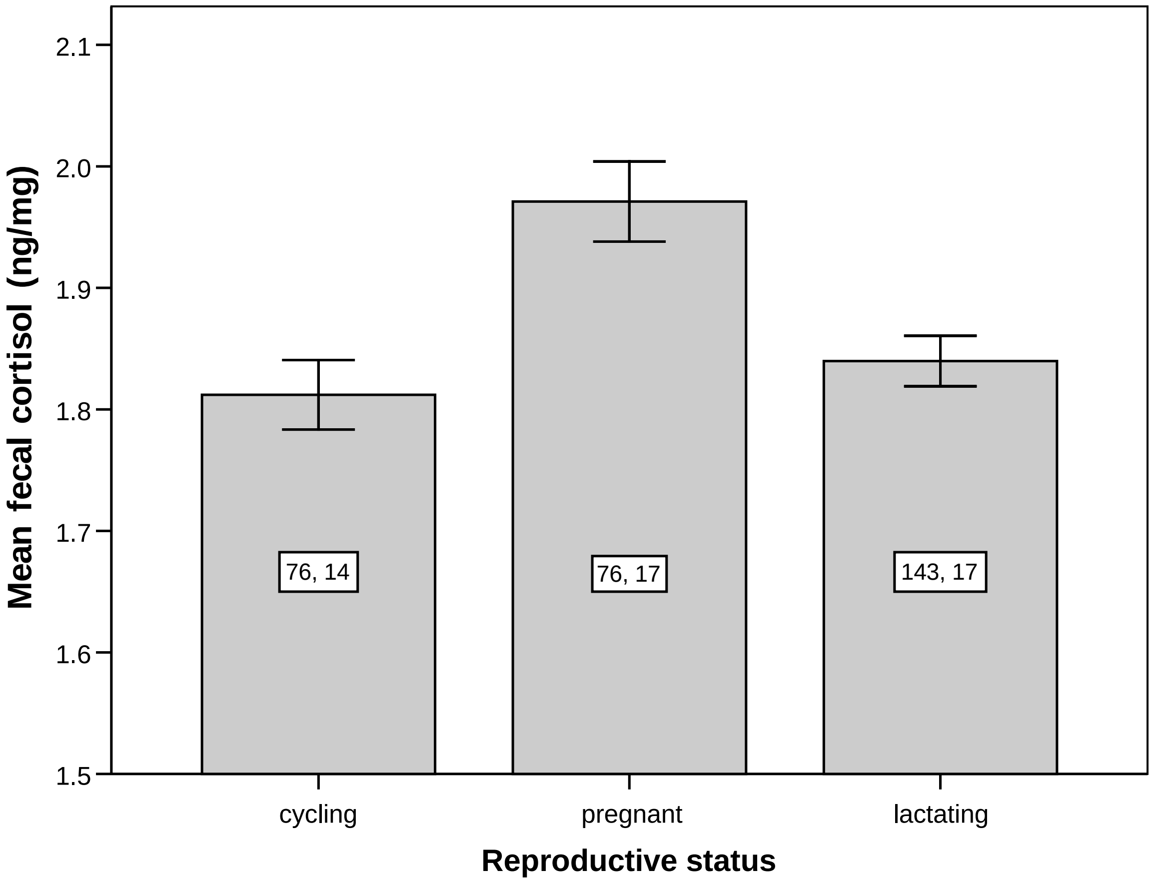
FIGURE LEGENDS

Figure 1. Mean +/- SEM fecal cortisol levels for female mandrills in different reproductive states. Text in boxes represents number of samples, followed by number of females contributing

Figure 2. Mean +/- SEM fecal cortisol levels for females across the reproductive cycle. 0 indicates parturition. Black bar represents cycling females (data may represent several cycles), grey bars represent pregnant females, white bars represent lactating females. Text in boxes represents number of samples, followed by number of females contributing



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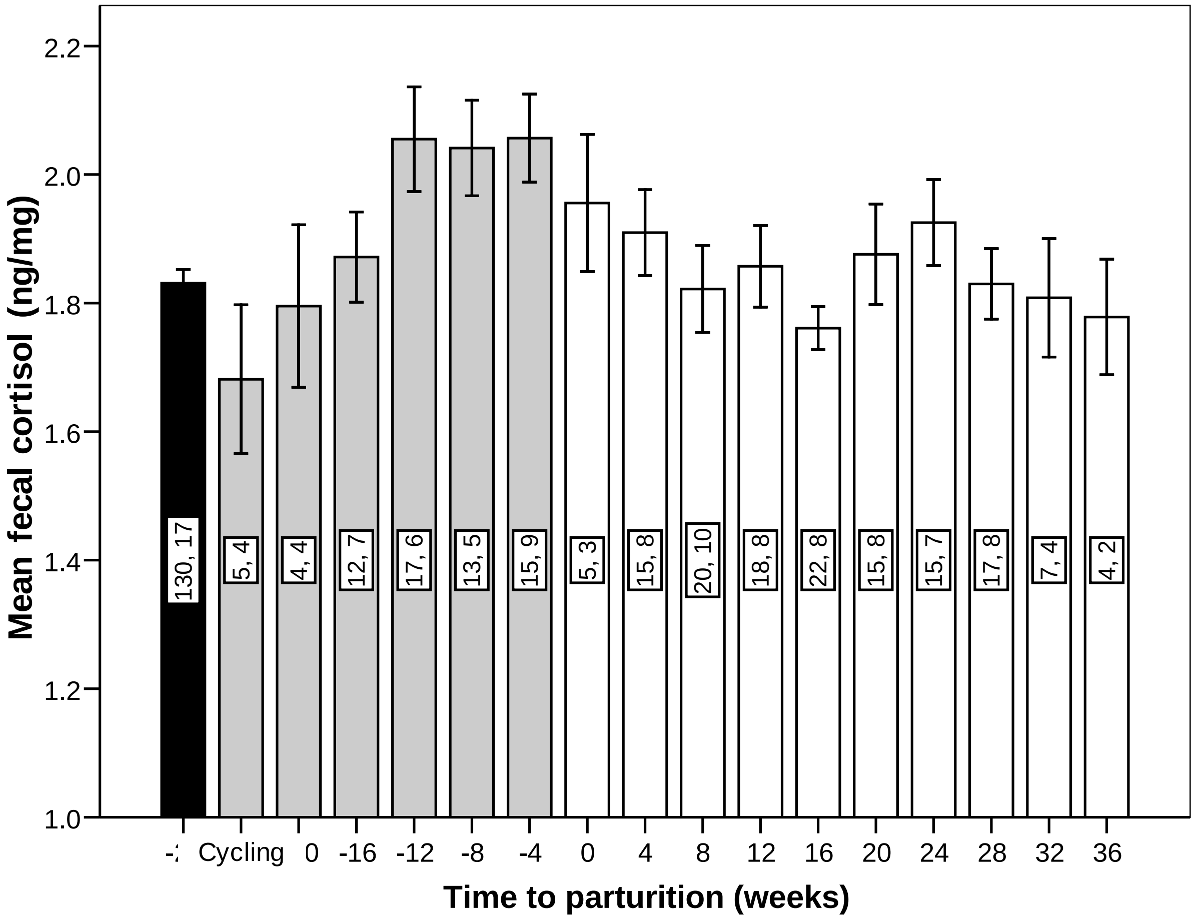

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