American Journal of Primatology 36:285–297 (1995)

Fecal Steroid Analysis of Ovarian Cycles in Free-Ranging Baboons

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This paper reports field and laboratory tests of serial sampling, solid phase extraction, and microradioimmunoassay methods for the collection, preservation, and analysis of fecal steroids. The field study was conducted in a troop of 87 yellow baboons (*Papio cynocephalus*) in the Tana River Primate Reserve, Kenya. Serial samples of four focal females and opportunistic sampling of 18 additional females over 22 days of sampling yielded a total of 62 samples, X = 3.1 ± 0.4/day, demonstrating the feasibility of regular field collection and extraction. Estradiol and progesterone concentrations in the field-extracted samples exhibited high recovery and statistically significant correlations ($P < 0.05$) with concentrations in the lab-extracted samples, suggesting that solid phase extraction could provide a useful alternative to freezing in sites where electricity or liquid nitrogen is not available. Tests of microradioimmunoassays demonstrated that these assays were sensitive, accurate, and precise when applied to the assay of fecal extracts, providing estimates of ovarian steroids that varied significantly with reproductive state. The demonstration that testosterone could be accurately and reliably assayed in fecal extracts suggests that these techniques also could be applied to the study of male reproductive function. Parallels between fecal profiles of cycling and pregnant baboons with patterns reported for serum steroids in baboons suggest that fecal steroids might be useful in distinguishing amenorrhea from early pregnancy in free-ranging baboons as well as in species lacking external indices of reproductive state.

Key words: *Papio cynocephalus*, fecal steroids, estradiol, progesterone, testosterone

INTRODUCTION

Conspicuous perineal swellings and coloration have facilitated field studies of reproductive function in baboons and a number of other cattarhine primates [Hrdy & Whitten, 1987]. Swelling and reddening of the sexual skin reflects estrogen

Received for publication July 21, 1993; revision accepted November 14, 1994.

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stimulation, whereas progesterone stimulus or estrogen withdrawal results in rapid deflation, providing a visible index of ovarian events [Collings, 1926; Hisaw, 1942; Gillman, 1940]. In baboons (*Papio cynocephalus*), 42–56% of ovulations occur in the 5 day period prior to detumescence [Wildt et al., 1977; Shaikh et al., 1982], facilitating the analysis of ovarian cycles. However, the sexual skin is a less specific index of ovarian events than hormonal concentrations [Robinson and Goy, 1986]. Because both estrogen decline and rising progesterone induce detumescence [Hisaw, 1942; Goy, 1979], follicular atresia cannot be readily distinguished from an ovulatory event [Robinson & Goy, 1986], a competent luteal phase cannot be distinguished from an inadequate one, and an acyclic condition cannot be easily distinguished from the early weeks of pregnancy [Wasser, 1983]. Moreover, as in other estrogen-responsive tissues, sex skin responses reflect tissue-specific factors such as steroid receptor concentrations [Kato et al., 1980; Ozasa & Gould, 1982] as well as circulating hormones. Divergences between circulating hormones and sex skin responses can be seen in the transitory sexual swellings that occur at puberty in rhesus macaques [Hisaw, 1942], the absence of sexual swellings during ovulatory cycles in some pigtail macaques [Hadidian & Bernstein, 1979], and individual variation in the timing of detumescence with respect to ovulation in baboons [Wildt et al., 1977; Shaikh et al., 1982]. These issues are becoming particularly relevant in the emerging field of primate socioendocrinology [Ziegler & Bercovitch, 1990], where questions such as the role of stress and nutrition in reproductive suppression, the contributions of anovulation, mating competition, and miscarriage to long interbirth intervals, and the role of mate choice and proximate determinants in situation-dependent receptivity encourage a more detailed understanding of reciprocal, ongoing hormone–behavior interactions than can be achieved by analysis of sexual swellings alone.

The need for simultaneous assessments of social behavior and endocrine function presents a dilemma for socioendocrinologists since many invasive procedures may disrupt social behavior or physiologic response [Raleigh & McGuire, 1990]. The development of methods for the extraction and assay of gonadal steroids from fecal material [Adlercreutz & Martin, 1976; Risler et al., 1987; Shideler et al., 1989, 1993, 1994; Ziegler et al., 1989; Strier & Ziegler, 1994] provides a noninvasive solution for those interested primarily in steroid–behavior interactions. However, application of these techniques to the study of free-ranging primates requires the development of a feasible and reliable field method for the collection and preservation of fecal samples. Wasser et al. [1988, 1991] have shown that ethanol can preserve estradiol (E_2) and progesterone (P_4) concentrations for up to 6 h and have used a solution of sodium azide in ethanol for short-term preservation of samples during field collection. However, long-term stability in these studies was achieved by storage in a freezer, a procedure that is not available in many remote field sites lacking refrigeration or access to liquid nitrogen. We have addressed this issue through the development of a method for the field extraction and preservation of fecal steroids that requires only a syringe, methanol, and water [Stavisky et al., 1991]. This paper provides a field test of steroid stability and recovery following field collection and extraction of fecal samples from a population of feral yellow baboons (*Papio cynocephalus cynocephalus*).

A second goal was to test the applicability of microradioimmunoassay techniques for the assay of fecal steroids [Stavisky et al., 1991, 1992, 1993]. These procedures have the advantages of 1) high specificity [Worthman et al., 1990; Stavisky et al., 1993], which may minimize the contributions of cross-reacting metabolites to fecal steroid determinations, 2) high sensitivity [Worthman et al., 1990], allowing the assay of even small quantities of steroids and facilitating

sample dilution to reduce the contributions of cross-reacting steroids, and 3) low cost, due to the smaller quantities of reagents required. Previous investigations found that E_2 and P_4 could be reliably measured by radioimmunoassay (RIA) in extracts of baboon feces and were significantly correlated with their concentrations in serum [Wasser et al., 1988, 1991]. Fecal profiles from two cycling baboon females exhibited E_2 peaks before or during detumescence and P_4 peaks between detumescence and menses, although considerable variability was apparent, particularly in fecal E_2 [Wasser et al., 1991]. Fecal profiles from three pregnant females showed a general tendency for increase in E_2 and P_4 over gestation, again with substantial variability within and among females [Wasser et al., 1991]. This paper extends these observations by comparison of fecal E_2, P_4, and testosterone (T) concentrations and profiles to reproductive state and sexual skin cycles in feral baboons.

MATERIALS AND METHODS

Animals and Sample Collection

Study site. The study site was the Tana River Primate Reserve in southeastern Kenya. The Reserve is situated on the lower Tana River floodplain and consists of savannah and several small gallery forests [Marsh, 1976, 1986]. The reserve contains approximately 10 baboon troops with an average of 90 members per troop. The study troop consisted of 87 members, with 25 females of reproductive maturity (≥5 years of age). Female reproductive histories were known from 1988, and ages were estimated from body weight, dental eruption, and time of menarche [Condit, in preparation]. Females were classified by reproductive state according to the following criteria: juvenile (18 months to 3 years), adolescent (over 3 years, premenarcheal), subadult (postmenarcheal, prior to first pregnancy), cyclic adult, (≥5 years, postmenarcheal and parous, sexual swelling observed during sample period), pregnant adult (≥5 years, postmenarcheal, persistent pink or red paracallosal coloration following a sexual cycle), and lactating adult (≥5 years, postmenarcheal and parous, presence of suckling infant, no sexual swelling observed during sample period). Cycle state of all females was assessed daily by rating perineal size and coloration, following Hausfater et al. [1983].

Field collection procedures. Four females who had exhibited regular perineal cycles in the previous months were selected for systematic, daily fecal collections during July and August 1990. These females were 1) a cycling adult female approximately 8.5 years of age, 2) a cycling subadult/adult female, 6.5–7 years of age, later determined to be in the conceptive cycle and early weeks of her first pregnancy, 3) a nonswelling subadult female, later determined to be in the second trimester of an unsuccessful pregnancy, and 4) a postmenarcheal adolescent female, approximately 6.5 years of age, who exhibited a temporary cessation in sex skin swellings during the sample period. Additional samples were collected on an ad libitum basis from the remainder of females in the troop; their ages and reproductive states are given in Table II. Samples were collected from focal and other females over a 5 week period, with samples acquired from one or more focal females on 20 of the 22 days on which collection was attempted. Samples (n = 38) from focal females represented 60% of the total sample (n = 63) collected with an average of 11 samples per focal female. Fecal samples were collected at the same time each day, following the first observed morning evacuation (06:00 to 06:30). Each sample was placed in an individually labeled Whirl Pak bag and sealed. These samples were extracted no later than 2 h after collection to prevent degradation of the steroids [Wasser et al., 1988].

Extraction Procedures

Extraction procedures. Duplicate or triplicate samples of 0.5 g were removed from each fecal sample. One sample was frozen for later tests of steroid recovery following laboratory extraction (focal females only). The second sample was dried in a gas oven to determine the dry weight, and the third was homogenized in 10 ml ethanol:acetone (8:2). A 10 ng aliquot of T was added to assess steroid recovery. An aliquot (4 ml) of the homogenate was filtered through a polytetrafluoroethylene (PTFE) syringe filter (0.2 μm; Millipore) to remove particulate matter. The filter was then washed with an additional 4 ml of ethanol:acetone. The filtrate was diluted 1:2 with filtered water and was passed through a Sep-Pak C18 cartridge (Waters Associates Inc, Milford, MA). After sample preparation, Sep-Pak C18 cartridges were primed with 2 ml of methanol, followed by 5 ml of water, based on the manufacturer's recommendations The cartridge was then washed with 2 ml of 0.1% solution of the preservative sodium azide to further reduce the chance for degradation [Shackleton & Whitney, 1980]. Each of the loaded cartridges was placed in a labeled, sealed plastic bag and stored in a freezer awaiting transport to the laboratory. Prior to RIA, each cartridge was washed with 3 ml of water, and the steroids were slowly eluted with 3 ml of methanol.

Radioimmunoassay (RIA) Procedures

E_2. The E_2 RIA followed the microassay procedures developed by Worthman et al. [1990], using reagents from the Estradiol-Quant-In Vitro ^{125}I RIA Diagnostic Test Kit (Leeco Diagnostics, Inc., Southfield MI) or from the Pantex Direct ^{125}I Estradiol 174M kit (Santa Monica, CA). Working buffer was 0.1% gelatin phosphate buffered saline (pH 7.4), achieved by heating 0.1 g gelatin in 100 ml Dulbecco's Buffer (Gibco, Grand Island, NY), at 45°C. In the Leeco version, the E_2 antiserum (Leeco) was diluted 1:4 in working buffer. E_2 standards (Leeco) were diluted 1:5 with the working buffer to give concentrations of 2–400 pg/ml. Additional controls in human serum base (Leeco) were diluted 1:5 in the assay buffer to yield a high and low E_2 control. The PEG-second antibody solution (Leeco) was diluted 1:2 with working buffer. Aliquots of fecal extracts were evaporated under nitrogen and reconstituted 1:1 in the working buffer. ^{125}I E_2 tracer (50 μl; Leeco) and diluted antiserum (100 μl) were added to aliquots (200 μl) of the standards, samples, and controls. After overnight incubation at room temperature, diluted second antibody (500 μl) was added, and the incubates were vortexed, incubated an additional 20 min at room temperature, and centrifuged at 1,500*g* for 60 min at 4°C in a Sorvall RT6000B with H1000B rotor (DuPont, Wilmington, DL). In the Pantex version, ^{125}I E_2 tracer (20 μl; Pantex) and antiserum (500 μl, diluted 1:8; Pantex) were added to aliquots (50 μl) of the standards (diluted 1:10 to give concentrations of 1–300 pg/ml; Pantex), samples, and controls (diluted 1:10; Pantex). After overnight incubation at room temperature, second antibody (500 μl, diluted 1:4) was added, and the incubates were vortexed, incubated 1 h at room temperature, and centrifuged at 1,500*g* for 1 h at room temperature. The supernatant was decanted, and the radioactivity of the precipitate was determined by 10 min counts in a RIASTAR gamma counter (Packard, Downer's Grove, IL) with RIASMART and Expert QC software.

P_4. The P_4 RIA followed the microassay procedures developed by Worthman and Stallings [unpublished] using reagents from the Pantex Direct ^{125}I Progesterone Kit for serum determinations. Working buffer was phosphate buffered saline (pH 7.4) with 0.1% bovine serum albumin and 0.1% sodium azide. The P_4 antibody (Pantex) was diluted 1:6 in working buffer. P_4 standards (Pantex) were

diluted 1:5 in working buffer to give concentrations of 0.04–16 ng/ml. Controls containing P_4 (Bio-Rad, ECS Division, Anaheim, CA) in a human serum base were also diluted 1:5 in working buffer. Aliquots of fecal extracts were reconstituted 1:1 in working buffer. ^{125}I P_4 tracer (100 μl; Pantex) and antiserum (100 μl) were added to 100 μl aliquots of the standards, samples, and controls. Each was vortexed and incubated overnight at room temperature. The following morning, undiluted second antibody (100 μl; Pantex), was added, and the incubates were vortexed, incubated for an additional 1 h at room temperature, and centrifuged at 1,500*g* for 1 h at 22°C. Following decanting of the supernatants, the radioactivity of the precipitate was determined by 10 min counts in the gamma counter.

T. The T RIA followed microassay procedures developed by Beall et al. [1992]. This protocol utilized reagents from the Leeco In Vitro ^{125}I RIA Diagnostic Kit for Testosterone (Leeco). Working buffer was 0.1% gelatin phosphate buffered saline (pH 7.4). T standards (Leeco) were diluted 1:10 in working buffer to give concentrations of 1–100 ng/dcl. Male and female serum controls (Leeco) were also diluted 1:10 in working buffer. The PEG second antibody was diluted 1:2 in working buffer. Aliquots of fecal extracts were reconstituted 1:1 in working buffer. ^{125}I T tracer (50 μl; Leeco) and antiserum (100 μl) were added to 100 μl aliquots of the standards, samples, and controls. Each was vortexed and incubated overnight at room temperature. The following morning, diluted second antibody (500 μl; Leeco), was added, and the incubates were vortexed and centrifuged at 1,500*g* for 20 min at room temperature (further research has demonstrated better statistical results when the incubates are centrifuged at 1,500*g* for 60 min at 4°C [Beall et al., 1992]). Following decanting of the supernatants, the radioactivity of the precipitate was determined by 10 min counts in the gamma counter.

Statistical Tests

Nonparametric tests were used for most comparisons since the distributions of some of the component groups departed significantly from normality, as indicated by the Kolmogorov-Smirnov test. The significance of differences between groups was tested with the Mann-Whitney U-test. Multigroup comparisons were made using the Kruskal-Wallis ANOVA on ranks with post-hoc comparisons between groups by Dunn's method. Correlations between variables were tested using linear regression. Statistical significance was assumed to be achieved at $P < 0.05$.

RESULTS

Feasibility of Fecal Collection From Known Individuals

The field trials demonstrated that fecal collection from known wild baboons was quite feasible. The assignment of samples to individuals was facilitated by collecting samples when the animals were still in their sleeping location, because the samples usually fell directly beneath them. Correct identification was assured by collecting only samples that had been seen to be excreted. Tree height did affect the condition of some samples, but only a few were unusable due to disintegration. An average of 3.1 ± 0.4 samples were collected each day from the entire group. Morning samples could be collected after it was light enough for individual identification and before the animals descended. Therefore, collection success was directly related to the time of descent from sleeping trees: when the animals were in the process of descending as sample collection began, only one or two samples could be collected, but as many as five samples could be collected when the animals remained in the sleeping trees for 15 min or longer after first light.

TABLE I. Accuracy and Precision of Fecal Steroid RIAs in *Papio cynocephalus*

Steroid	Sensitivity[a]	Accuracy (n)	Paral-lelism[b]	Tube error (n)	Intraassay CV (%)[c] Low (n)	Intraassay CV (%)[c] High (n)	Interassay CV (%)[d] Low (n)	Interassay CV (%)[d] High (n)
E_2								
Leeco	0.36 pg/ml	97.8%; y = 0.87x + 27.5; r = 0.985 (5); $P = 0.002$	Yes	2.1 (10)	14.4 (5)	12.8 (5)	8.3 (6)	12.9 (6)
Pantex	0.36 pg/ml	91.2%; y = 1.20x − 1.1[e]; r = 1.00 (5); $P < 0.001$	Yes		3.2 (4)	2.5 (3)	2.7 (3)	
P_4	0.006 ng/ml	117.9%; y = 1.00x + 0.6; r = 0.992 (8), $P = 0.0001$	Yes	1.6 (10)	1.8 (6)		6.3 (3)	4.0 (3)
T	0.1 ng/dl	106.5%; y = 0.83x + 5.4; r = 0.995 (5), $P = 0.003$	Yes	3.4 (10)	19.7 (5)	17.9 (5)	16.8 (9)	18.9 (9)

[a]Defined as the quantity of unlabeled hormone required to inhibit binding of tracer by an amount equal to two standard deviations below the mean binding in the absence of unlabeled hormone.
[b]The regression equations obtained for dilution sequences were y = 0.96x − 18.6x for E_2L (r = 0.998, $P = 0.002$; n = 4), y = 1.01x − 0.31 for P_4 (r = 0.999, $P = 0.0001$; n = 5), and y = 1.18x − 0.881 for T (r = 0.998, $P = 0.050$; n = 3).
[c]Within assay coefficient of variation of fecal sample pools.
[d]Between assay coefficient of variation of serum controls.
[e]With inclusion of the highest standard (300 ng/ml): y = 2.52x − 32.55; see text.

Field Tests of Steroid Recovery

Steroid recovery from field-extracted samples was assessed by comparison of E_2 and P_4 values in the field- and laboratory-extracted samples. E_2 concentrations in the field-extracted samples were 115.1 ± 16.5% (n = 22) of lab-extracted samples, and P_4 concentrations were 72.6 ± 9.4% (n = 22) of lab-extracted samples. Concentrations in the paired field-extracted and field-frozen, lab-extracted samples were then compared to assess the reliability of the field extraction process. E_2 concentrations obtained from RIA of field-extracted samples were highly correlated (r = 0.994, y = 0.919x + 0.004, $P < 0.0001$; n = 22) with the values obtained from the lab-extracted samples, suggesting that the field procedures yielded a steroid extract that reliably reflected fecal steroid concentrations present in the original sample. P_4 concentrations in the field-extracted samples also were significantly correlated with concentrations in the lab-extracted samples, although the slope of the regression equation indicated a lower recovery (r = 0.669; y = 0.342x + 0.236, $P = 0.001$; n = 22). To reduce this bias, the P_4 values from the lab-extracted samples were used for focal females, and the concentrations in the other samples were corrected by the mean recovery (0.7) for P_4.

RIA Validity

Tests of sensitivity, accuracy, and precision indicated that the microRIAs could be reliably applied to baboon fecal extracts (see Table I). Some variability was apparent across manufacturers of assay reagents. The Pantex-based E_2 assay exhibited greater precision than the Leeco-based assay, but accuracy was reduced at the highest E_2 concentration by a marked amplification of dose (see note to Table II). To avoid this bias, samples were assayed for E_2 at a 1:10 dilution.

Comparison of Fecal Steroid Profiles to Perineal Cycle State

Table II presents the fecal E_2 and P_4 values observed and their relationship to reproductive status assessed by reproductive histories and sex skin cycles in the

TABLE II. Relation of Fecal Steroid Concentrations to Reproductive State in Female Baboons

Reproductive state	Age	Samples /females	E_2 ng/gm	P_4 ng/gm
Immature				12.9 ± 2.0
Premenarcheal	Juvenile	2/2	1.3 ± 0.4	13.7 ± 5.4
Premenarcheal	Adolescent	3/2	0.7 ± 0.2	17.3 ± 4.2
Postmenarcheal	Subadult	8/4	0.6 ± 0.3	9.7 ± 2.1
Lactating	Adult	9/8	8.2 ± 4.6	78.5 ± 41.2
Cycling	Adult	10/1	3.9 ± 2.8	108.8 ± 53.3
Pregnant	Adult	22/5	13.9 ± 8.3	83.0 ± 26.5

sampled females. P_4 concentrations varied significantly with reproductive state ($H = 18.349$, $df = 3$, $P < 0.001$) and were higher in samples from cycling ($Q = 3.89$, $P < 0.05$) and pregnant females ($Q = 3.428$, $P < 0.05$) than in samples from immature females. No significant relationship was apparent for E_2 concentrations.

Steroid concentrations in samples from eight lactating females were in the juvenile range, with the exception of two females who exhibited values in the range of those seen in cycling females (see Table III). There was no obvious relationship to the duration of lactation; in fact, one of the two high values occurred within 1 month of parturition. Since none of the females exhibited sexual swellings during this period, the meaning of these high values is unclear.

E_2 concentrations increased with stage of gestation ($r = 0.710$, $y = 0.627x - 19.553$, $P < 0.001$), and especially high values were observed 1 week before parturition (see Table IV). In contrast, P_4 concentrations were not associated with gestational stage ($H = 1.028$, $P = 0.794$); in fact, the highest P_4 values were seen between 41 and 80 days.

Steroid Profiles

Profiles from the luteal phase and early follicular phase of two successive conceptive cycles are depicted in Figure 1. Fecal P_4 exhibits a luteal phase elevation, peaking at 9 days and falling to baseline values 16 days after maximal tumescence, matching reported profiles of serum P_4 in cycling baboons [Stevens et al., 1970; Wildt et al., 1977; Kling & Westfahl, 1978]. Luteal phase concentrations (133.7 ± 75.8 ng/g) surpass follicular phase values (51.7 ± 10.9 ng/g), but this difference is not statistically significant. E_2 and T rise in conjunction with P_4 and with the onset of tumescence in the subsequent cycle. In contrast with this profile, uniformly low E_2 (0.4–1.9 ng/g) and P_4 (7.3–14.7 ng/g) were observed in a subadult female who was not swelling during the sample period.

The relation of fecal steroids to gestation stage and paracallosal skin color changes is depicted in Figures 2 and 3, showing two nulliparous females (6.5–7.5 years of age [Condit, in preparation] in their first pregnancy. A clearly pink paracallosum denotive of pregnancy was not yet evident in these females, but changes in the normal gray-black paracallosal coloration were noted following peaks in E_2/P_4 excretion. Figure 2 depicts the luteal phase and subsequent weeks of a conceptive cycle that culminated in parturition 175 days after the recorded sex swelling deflation. Given a gestation length of 175–185 days [Hausfater, 1975], it is likely that this profile reflects the first 6 weeks of gestation. T rises to a peak at day 12, resembling that seen at day 9 in the nonconceptive cycle, whereas peaks in P_4 and E_2 can be seen at day 42. This profile resembles serum profiles of baboon

TABLE III. Fecal E_2 and P_4 in Lactating Female Baboons

Female	Lactation day	E_2 ng/gm/P_4 ng/gm	
CO	9	0.1	12.2
	26	43.0	297.0
ML	197	0.7	12.8
HQ	285	1.2	17.4
EU	335	21.9	294.4
MU	346	1.9	11.6
NY	413	0.8	6.1
LS	463	1.7	39.4
KM	538	1.0	15.3

TABLE IV. Fecal E_2 and P_4 in Baboon Pregnancy

Gestation day	Samples/females	E_2 ng/gm	P_4 ng/gm
1–40 days*	9/1	1.1 ± 0.3	66.4 ± 24.6
41–80 days	10/2	6.9 ± 3.4	112.2 ± 49.0
121–160 days	2/2	16.5 ± 15.5	54.1 ± 41.8
161–185	1/1	174.1	44.1

*Days from ovulation, defined as last day of maximum tumescence [Wildt et al., 1977].

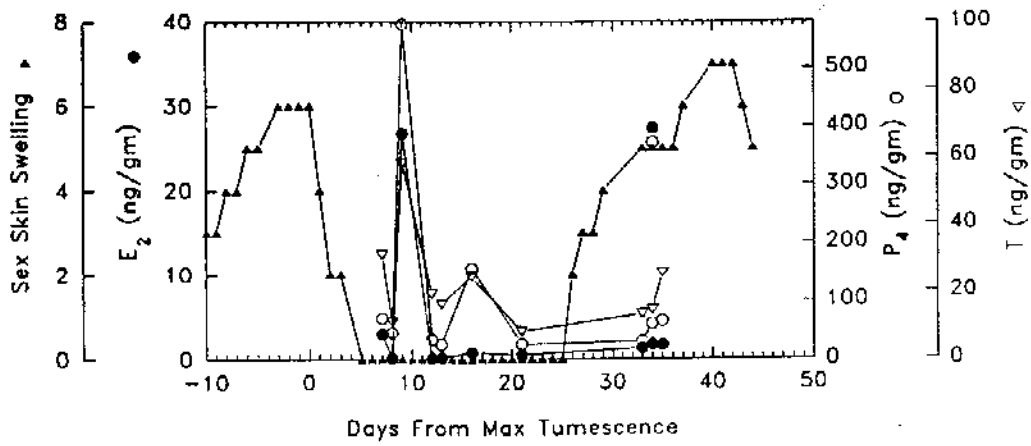

Fig. 1. Fecal E_2 and P_4 compared to perineal swelling size across the menstrual cycle of a female baboon.

gestation showing elevations in T on days 10–19 [Hodges et al., 1984] and peaks in P_4 on day 37 [Fortman et al., 1993; Albrecht & Townsley, 1978]. In Figure 3, depicting weeks 8–13 of an unsuccessful pregnancy, P_4, E_2, and T rise and fall between 63 and 68 days, resembling profiles of serum E_2 showing a sustained elevation after day 58 [Fortman et al., 1993; Albrecht & Townsley, 1978] that plateaus around day 72 [Fortman et al., 1993]. In the fecal profile, E_2 subsequently declines to baseline values over the next 2 weeks, followed by loss of paracallosal skin coloration, as in serum profiles of spontaneously aborting baboons [Fortman et al., 1993].

DISCUSSION

This study has demonstrated the feasibility of field collection and extraction. Although samples were extracted within 2 h of collection in these investigations,

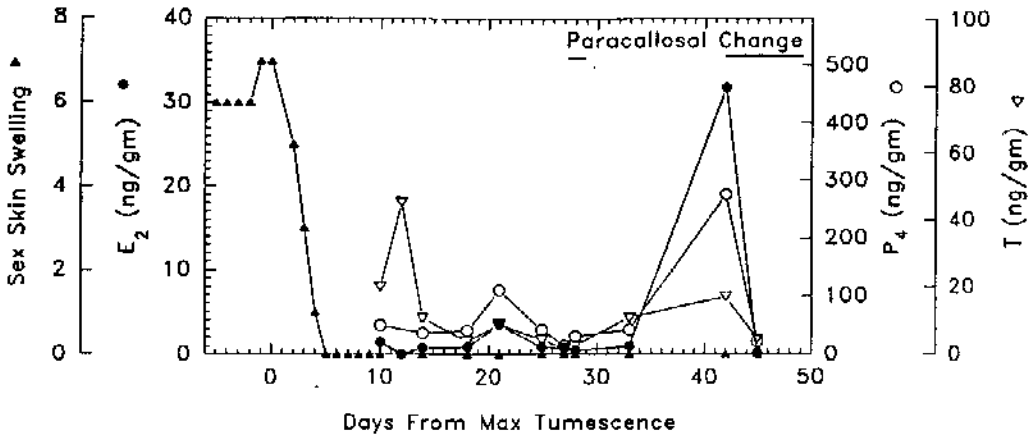

Fig. 2. Profiles of fecal E_2, P_4, and T during the first 6 weeks of gestation in a female yellow baboon. Horizontal lines denote the periods during which a pinkish hue was observed in the upper quadrant of the paracallosal skin.

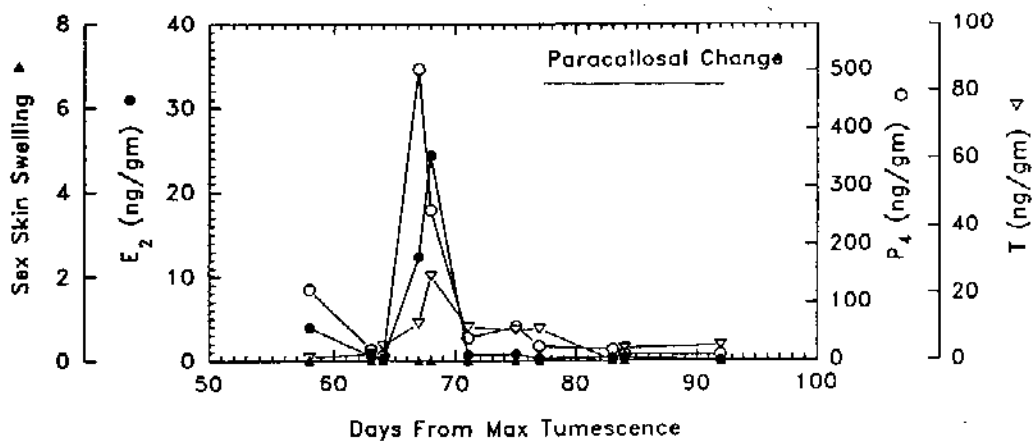

Fig. 3. Profiles of fecal E_2, P_4, and T during weeks 8–13 of an unsuccesful pregnancy in a female baboon.

Wasser et al.'s work [1988] has shown that samples can be temporarily preserved in ethanol, facilitating extraction at the end of the field day if observation schedules preclude immediate extraction. Tests of stability show that solid phase cartridges can be used to preserve the extracted steroids in a field situation. Concentrations of E_2 in the field-extracted samples showed a high positive correlation with concentrations in lab-extracted samples. We are uncertain of the cause of the lower correlation observed for P_4, but it may reflect less efficient extraction in the absence of a motorized homogenizer, a problem that could be obviated by a battery-operated device. These determinations suggest that solid phase extraction could provide a useful alternative to freezing in sites where electricity or liquid nitrogen is not available.

The microRIAs employed here are sensitive, accurate, and precise, providing estimates of ovarian steroids that predictably reflected reproductive state. Peak E_2 and P_4 concentrations in the Tana females were similar to peak concentrations reported by Wasser et al. [1991] for cycling baboons in Mikumi National Park, Tanzania, although he observed somewhat higher P_4 values in pregnant females.

Intersite comparisons of this sort, along with interindividual comparisons, may help to address concerns about the potential confounding effects of dietary variation on fecal steroid concentrations [Shideler et al., 1994]. Wasser et al. [1991] reported variable and inconsistent fluctuations in fecal E_2, a problem which we found could be minimized by a tenfold dilution of extracts in RIAs. These patterns were attributed to the baboon's predominantly urinary excretion of E_2, which seems an unlikely explanation. Even the low levels of E_2 found in baboon feces are an order of magnitude higher than those found in serum and should be accurately measured by the available RIAs. A proportionately low level of fecal excretion could be a problem if it were associated with fluctuations in the rate of fecal excretion. Enterohepatic recirculation could provide more opportunity for such fluctuations to occur through the influence of dietary components such as fat or fibre [see Adlercreutz, 1990]. However, the lower urinary excretion rate of P_4 in baboons suggests that it is the ovarian steroid most subject to enterohepatic circulation in baboons [Kulkarni et al., 1970]. Because this steroid appears to produce more, rather than less, consistent results, the predominantly urinary excretion of E_2 in baboons does not seem to be a likely explanation for its variable concentrations. A more likely explanation is the variable contributions of cross-reacting metabolites. The E_2 antibody employed by Wasser et al. [1988, 1991] exhibits a 2% cross-reactivity with 17α-hydroxy-P_4 which could result in a significant contribution of this steroid metabolite to measures of E_2, particularly during the luteal phase when fecal progestin concentrations are 10–20 times higher than fecal estrogen concentrations. The antibody employed here exhibits much lower cross-reactivity to metabolites such as 17α-hydroxy-progesterone, but it does cross-react with catechol estrogens which are present in the fecal samples of some baboon females in high concentrations [Stavisky et al., 1993; Whitten et al., 1993; Stavisky, 1994]. The instability of these estrogens in the absence of antioxidants along with individual differences in metabolism could result in variable estimates of E_2 dose. This cross-reactivity could also contribute to the variability that Wasser et al. [1991] observed if their E_2 antibody is characterized by a similar cross-reactivity.

The patterns of fecal steroids observed during pregnancy are of particular interest from the standpoint of applicability to species with sexual swellings. Although changes in paracallosal skin can be used to detect pregnancy in baboons, the 1–2 month delay in these changes [Hausfater et al., 1983; Wasser, 1983], combined with individual variability in their expression, may impede the detection of early pregnancy. The rate of spontaneous abortion estimated from pregnancies detected from paracallosal skin changes in wild baboons (10% [Altmann et al., 1988]) is considerably less than the rate estimated from hormonal data on captive baboons (60% [Kuehl et al., 1992]; 23% [Fortman et al., 1993]). Either conditions of captivity elevate abortion rates or many early unsuccessful pregnancies are missed in the field. All of the spontaneous abortions in captivity were observed at 19–48 days of gestation, suggesting that most of these pregnancies would have been too early to be detected by changes in paracallosal skin. In either case, it would appear that comparable hormonal data on wild and captive baboons, obtained with a noninvasive method, could provide some important insights into the determinants of successful pregnancy in baboons.

Concentrations and profiles of fecal steroids in pregnant females paralleled reports of serum profiles of gestation in baboons. Cercopithecoids do not exhibit the early sustained elevations in E_2 and P_4 seen in hominoid pregnancy [Bielert et al., 1976; Gordon et al., 1991; Fortman et al., 1993]. In baboons, serum P_4 exhibits only a transitory elevation between 24 and 51 days of gestation with a peak on day 37,

corresponding to peak chorionic gonadotropin secretion, whereas E_2 begins a sustained elevation after day 58 [Fortman et al., 1993; Albrecht & Townsley, 1978]. These secretory patterns appear to be reflected in the elevations in fecal P_4 and E_2 observed on days 42 and 67–68 of gestation and the elevations in fecal E_2 observed in samples from the last trimester. Elevations in fecal T on day 12 also parallel reports of fourfold elevations of serum T on days 10–19 [Hodges et al., 1984] reflecting the onset of increased chorionic gonadotropin secretion at this time [Hodges et al., 1984; Fortman et al., 1993]. Because deficits in chorionic gonadotropin secretion are implicated in spontaneous abortions in baboons [Kuehl et al., 1992; Fortman et al., 1993], profiles of T and P_4 could be useful in monitoring early pregnancy and spontaneous abortion. Although the significance of these potential parallels will have to be tested by more frequent serial sampling of fecal, and serum, steroids over a larger number of females, these preliminary obsevations suggest that the application of fecal steroids for the early detection of pregnancy in baboons warrants further investigation.

The predictable nature of the patterns observed here suggests that fecal steroid analysis has potential as a tool in the study of reproductive function in species with sexual swelling as well as those without sexual skin. Application of fecal steroids to monitor luteal phase competence, conception, and the early stages of pregnancy can provide important insights into the influence of social stress and nutrition in female reproductive success. Moreover, the demonstration that T can be reliably and accurately measured in fecal extracts provides an opportunity for the assessments of gonadal function in males as well as females. These techniques open doors for investigation of the reproductive biology of free-ranging primates, without the cost or risk of blood draws or other intrusive procedures, to increase our knowledge of reproductive function and reproductive status as a corollary of behavioral research.

CONCLUSIONS

1. Baboon fecal steroids can be collected and extracted under field conditions.
2. Solid phase extraction cartridges provide a convenient and reliable method for the field preservation of fecal E_2 and P_4.
3. P_4 and E_2 can be accurately and reliably assayed with microRIAs in extracts of baboon feces with concentrations reflecting reproductive state (P_4) and gestation (E_2).
4. T can be accurately and reliably measured in baboon feces, opening the possibility for field assessments of male endocrine function.

ACKNOWLEDGMENTS

We thank the Office of the President, Kenya, for permission to conduct this research (OP.13/001/20C 240/13) and the National Museums of Kenya and the Institute of Primate Research, Kenya, for technical support and assistance at the Tana River National Primate Reserve, with special thanks to Dr. M. Isahakia, Prof. S Njuguna, and Dr. J. Else. We thank Hassan Jilo for field support. This research was supported by Emory University (P.L.W.), The Emory University Research Committee (P.L.W., E.O.S.), The Mellon Foundations (R.S.), National Science Foundation grants BNS-8921106 (E.O.S.) and SBR-9304633 (P.L.W.), and National Institutes of Health grant RR00165 (Yerkes) from the National Center for Research Resources. The Yerkes Center is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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