American Journal of Primatology 32:95–108 (1994)

Monitoring Female Reproductive Function by Measurement of Fecal Estrogen and Progesterone Metabolites in the White-Faced Saki (*Pithecia pithecia*)

S.E. SHIDELER[1], A. SAVAGE[2], A.M. ORTUÑO[3], E.A. MOORMAN[1], AND B.L. LASLEY[1]
[1]Institute of Toxicology and Environmental Health and the California Regional Primate Research Center, University of California, Davis; [2]Roger Williams Park Zoo, Research Department, Providence, Rhode Island; [3]Universidad Nacional, Escuela de Medicina Veterinaria, Heredia, Costa Rica

A simple method for extracting and measuring ovarian steroids in feces is applied to the ovarian cycle, pregnancy, parturition, and period of lactational amenorrhea in *Pithecia pithecia*. Small amounts of wet, unmixed feces were combined with a modified phosphate buffer, shaken, centrifuged, and decanted, and the supernatant was directly measured for estrogen and progesterone metabolites by enzyme immunoassays. Urinary estrogen and progesterone metabolite measurements were compared to paired fecal measurements to determine the degree to which fecal hormone levels detected the same ovarian events as urinary measurements. The correlation coefficients for the relationship between urinary and fecal hormones for individual animals studied (n = 5) were found to be statistically significant in every case except one sexually immature animal. The application of the method presented here demonstrates that simple solubilization and non-radiometric measurement of ovarian steroids excreted in feces reliably reflect reproductive events in *Pithecia pithecia*.

Key words: urinary steroids, fecal steroids, ovarian cycle, pregnancy, enzyme immunoassays

INTRODUCTION

There has been an increasing interest in the development of laboratory methods to measure steroid hormones in the excreta of free-ranging animals. In recent years, many researchers have developed various technologies that permit the monitoring of aspects of female reproductive status through measurement of fecal hormones [Bamberg et al., 1991; Desaulneirs et al., 1989; Kirkpatrick et al., 1990, 1991; Lasley & Kirkpatrick, 1991; Messier et al., 1992; Royal Rotterdam Zoological and Botanical Gardens, 1992]. In primatology, Wasser and his associates have pioneered the quantification of steroid hormones in primate feces [Wasser et al., 1986, 1988, 1991, 1992a,b; Risler et al., 1987].

Received for publication January 11, 1993; revision accepted July 10, 1993.

Address reprint requests to Susan E. Shideler, ITEH, University of California, Old Davis Road, Davis, CA 95616-8615.

The present report describes the application of a simple, non-radiometric technique of monitoring fecal ovarian hormone metabolites that has been demonstrated to accurately assess the reproductive cycles of *Macaca spp.* [Shideler et al., 1993]. Recently a colony of *P. pithecia* has been made available for studies of their reproductive biology and behavior in order to enhance propagation and management in captivity, to develop techniques of hormonal analysis that can be applied to field studies, and to compare the reproductive biology and behavior of sakis in the wild to that of those observed in captivity. This report demonstrates the feasibility of obtaining similar physiological information from the collection of fecal as compared to urine samples so that fecal samples may be used as an alternative to urine collection in the field. The urinary and fecal ovarian steroid dynamics of sexual maturation, the estrous cycle, pregnancy, parturition, and lactational amenorrhea in captive white-faced sakis are compared.

MATERIALS AND METHODS

Animals

Five female *Pithecia pithecia* females, aged 18–120 months (18, 18, 47, and 65, and one wild-caught female estimated to be 120 months), were housed in cages between 7.5 m × 2.5 m × 4.0 m and 2.4 m × 3.0 m × 3.0 m. Animals were maintained on a 12L:12D light regimen at 78°F, and relative humidity was 65%. Animals had ad libitum access to water and were fed twice a day. The morning diet consisted of New World Primate Chow 5040 (Purina Mills, Inc., St. Louis, MO), ZuPreem primate diet (Hill's Pet Products, Topeka, KS) and vitamin and calcium supplements. The afternoon diet consisted of New World Primate Chow 5040, fresh fruits, vegetables, nuts, eggs, mealworms, and vitamin supplements. Adult females lived with adult males and their offspring born during the study. Preadolescent and adolescent females were caged alone.

Sample Collection and Preparation

Urine and fecal samples were collected each morning for a maximum of 1 year and 10 months, from 1990 to 1992, labeled, and frozen without preservatives at −20°C until assayed. Using a technique similar to Ziegler et al. [1987], first morning void urine and feces were collected daily from each female without restraint, isolation, or handling. Animals were habituated to the presence of humans entering their cages. At 0800 h, colony lights were turned on and a sample collector entered the cage and fed the female 10–15 sunflower seeds. First morning void urine was collected directly in a polyproplyene container hand-held under the female subject. A second container was held under the female to collect fecal material. Females urinated and defecated within 5–20 min. Following sample collection, females were rewarded for their cooperation with more sunflower seeds.

To assay, frozen urine samples were allowed to thaw and were diluted 1:50 in distilled water. Forty microliters (0.040 ml) of diluted urine were measured directly by the estrone conjugate enzyme immunoassay (E_1C EIA) and 0.020 ml were measured directly by the pregnanediol-3-glucuronide enzyme immunoassay (PdG EIA). For the solubilization of fecal samples, a predetermined mass (0.25 g) of untreated, freshly frozen, wet fecal samples were placed into test tubes and 2.5 ml of modified phosphate buffer added (0.1 M, pH 7.0, 0.1% BSA, with .05% Tween 20 and 20% HPLC grade methanol). Samples were shaken for 24 h, then centrifuged (0°C) at 600–800g force for 10 min. The supernatant from the methanol solubilization was decanted into clean test tubes and the residual pellets were discarded. Twenty microliters (0.020 ml) and .040 ml of undiluted supernatant were directly measured by PdG and E_1C EIAs, respectively. Using 0.25 g feces in 2.5 ml solu-

bilizer, recoveries for E_1C were 40.54% (SD ± 5.04, n = 12) and for PdG were 32.20% (SD ± 2.38, n = 12).

To determine the proportion of conjugated and unconjugated steroids in feces detected by present assay systems used, the following experiment was performed. Aliquots of the supernatants from the methanol-buffer solubilization of daily fecal samples from the follicular/luteal phase (n = 5 samples, from consecutive days from animal 1), early pregnancy (n = 5 samples, from consecutive days, animal 1, approximately 30 days pregnant), and adolescence (n = 5, from consecutive days, animal 5) were extracted with 5 volumes of ethyl ether, vortexed for 1 min, frozen in a bath of methanol poured over dry ice, decanted, air-dried, and reconstituted in assay buffer (estrone conjugate radioimmunoassay: tris buffer, 0.1 M, pH 8.4; pregnanediol-3-glucuronide radioimmunoassay: phosphate buffer, 0.1 M, pH 7.0, 0.1% BSA) or 40% acetonitrile:60% distilled water. Both the buffer reconstituted ether extracts (unconjugated steroids) and the aqueous residuals (conjugated steroids) of these samples were measured by radioimmunoassay for estrone conjugate (E_1C) and pregnanediol-3-glucuronide (PdG) concentrations as previously described [Mitchell et al., 1983; Shideler et al., 1983; Monfort et al., 1987]. Sample values for ether extracts and residuals were combined for an estimated total daily measurement for the cycle, early pregnancy, and adolescent samples. The ether extract and residual values were also averaged to determine the percent of the total measurement that was obtained by either measurement and to estimate differences in excretion routes (or conjugation) between cycles, early pregnancy, and adolescent sampling intervals.

High Performance Liquid Chromatography (HPLC)

To evaluate the presences of fecal estrone conjugates measurable by the E_1C EIA and the conjugates measured by the PdG EIA, co-chromatography was performed in the following manner. Tritiated estradiol, estrone, and progesterone were added to extracted fecal samples (n = 4, from two adult females, each contributing one approximately 30 days pregnant and one cycling sample) that had been filtered (0.45 μm syringe filters: Acrodisc, Gelman Sciences, Ann Arbor, MI), dried down under a nitrogen stream, and reconstituted in 40% acetonitrile:60% distilled water. Samples were injected (100 μl) on to a Vydac gradient HPLC column previously described [Shideler et al., 1993, in press]. An aliquot of the eluates was taken for counting the tritium control peaks: the remaining eluates were reduced to dryness, reconstituted in EIA buffer, and assayed by EIAs described below.

Assays

Estrone conjugates were measured in urine and feces by an E_1C EIA previously characterized [Shideler et al., 1990; Munro et al., 1991]. Pregnanediol-3-glucuronide was measured in urine and feces employing a PdG EIA also characterized previously [Munro et al., 1991]. Urine samples were indexed by creatinine to compensate for variations in individual urine sample concentration, using Taussky's method [Taussky, 1954].

The inter-assay coefficient of variation for the E_1C EIA was 9.72% and 11.55% at 24% (n = 237) and 48% (n = 225) binding, respectively, and the 50% binding of the standard curve (n = 62) averaged 48 pg/well. The intra-assay coefficient of variation ranged from 2.81%–5.94% when four replicates per assay were analyzed (n = 10 assays). E_1C EIA of serial dilutions of urine and fecal samples exhibited a response parallel to the E_1C standard curve. For the PdG EIA, the inter-assay coefficient of variation was 19.14% at 64% binding (n = 91) and 16.67% at 38%

binding (n = 82). The intra-assay coefficient of variation ranged from 0.35–4.32% when four replicates per assay were analyzed (n = 12 assays). The 50% binding of the PdG EIA standard curve averaged 0.39 ng/well (n = 57). PdG EIA of serial dilutions of urine and fecal samples showed a dose-response parallel to the PdG standard curve.

To estimate the proportion of conjugated to free steroid in samples from different reproductive states, one radioimmunoassay each for E_1C and PdG were employed in this study. The control values for those assays were consistent with the values obtained for interassay coefficients of variation previously reported [Lasley et al., 1985]. The historic interassay coefficients of variation for these assays in this laboratory have been 22.2% and 16.4% at 85% and 44% binding, respectively, for the PdG RIA, and 9.9% and 8.1% at 32% and 16% binding for the E_1C RIA.

Statistics

Standard descriptive and sampling statistics were used to summarize results. Pearson product-moment correlation coefficients for the relationships between urinary and fecal hormone values were calculated for samples from both the urine and fecal compartments and were aligned by calendar day on which samples were collected. The highest significance level given is $P < .01$ although several tests gave much lower P values.

RESULTS

After HPLC separation [Shideler et al., in press], saki fecal sample eluates were assayed using the E_1C and PdG enzyme immunoassays. The results obtained for cycling and early pregnancy samples produced similar chromatograms with differences only in the magnitude of the peaks. The early pregnancy sample chromatogram depicted in Figure 1 shows the PdG EIA immunoreactivity and revealed one large peak with a declining, more polar shoulder (fractions 42–53) that partially overlapped the tritiated progesterone peak (fraction 46). An immunoreactive peak (fractions 19–23) also was observed when eluates were assayed by the E_1C EIA (Fig. 2). Tritiated estradiol eluted at fraction 18 and tritiated estrone eluted at fraction 20.

The percent unconjugated estrone measured by radioimmunoassay in fecal extracts after ether extraction was 78.77% for cycle samples, 84.71% for pregnancy samples, and 81.12% for adolescent samples. Percentages obtained from radioimmunoassay values for unconjugated progesterone metabolites were 27.72% for the cycle samples, 61.67% for pregnancy samples, and 28.55% for adolescence.

The average r values for paired comparisons of urinary and fecal PdG metabolite levels were 0.55 (SD = 0.30). For individual animals, all correlations were statistically significant at $P < .01$ with the exception of animal 3 (animal 1: $r = 0.79$, n = 467, df = 465; animal 2: $r = 0.77$, n = 203, df = 201; animal 3: $r = 0.04$, n = 206, df = 204; animal 4: $r = 0.58$, n = 122, df = 120; animal 5: $r = 0.55$, n = 67, df = 65). The average r values for paired comparisons of urinary E_1C levels to fecal E_1C levels were 0.58 (SD = 0.31) For individual animals, all correlations were statistically significant at $P < .01$ with the exception of animal 3 (animal 1: $r = 0.64$, n = 467, df = 465; animal 2: $r = 0.50$, n = 203, df = 201; animal 3: $r = 0.08$, n = 206, df = 204; animal 4: $r = 0.76$, n = 122, df = 120; animal 5: $r = 0.90$, n = 67, df = 65).

Figures 3 and 4 illustrate the estrogen and progesterone metabolite excretion profile measured in urine and in feces in one female white-faced saki (animal 1) from one pregnancy and birth of a live female offspring (approximately days

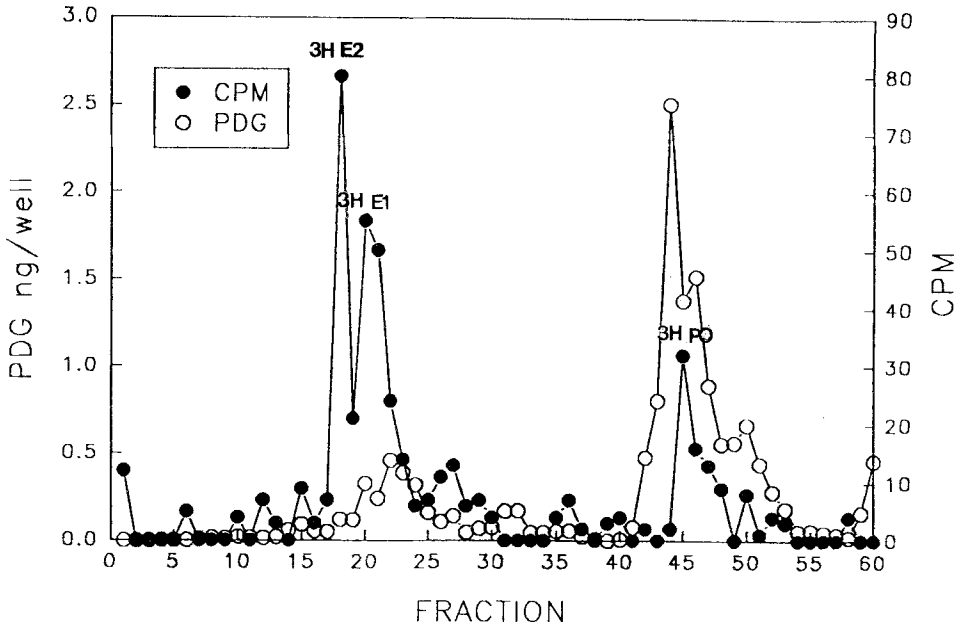

Fig. 1. Co-chromatography on a *Pithecia pithecia* fecal sample with high progesterone content as measured by PdG EIA (> 5.0 μg/mg Cr). Tritiated estradiol, estrone, and progesterone (^{3}H E_2, ^{3}H E1, and ^{3}H Po) are depicted by closed circles, and their units of measurement are on the right ordinate as counts/minute (CPM). Immunoreactive pregnanediol-3-glucuronide is represented by open circles, and the units of measurement are in nanograms/well (ng/well) on the left ordinate. The abscissa represents fractions collected on a 35% acetonitrile:65% dH_2O gradient column at a 1.0 ml/min flow rate and 0.5 ml fractions [Shideler et al., 1993, in press].

1–150), through lactational amenorrhea, the resumption of cycling (approximately days 345–450), conception, pregnancy, and live birth of a male offspring (approximately days 450–610), spanning 632 days of sample collection. These profiles are representative of the ovarian cycles and pregnancies of the three adult females studied. Figure 3 depicts the pattern obtained from measurements of urinary E_1C and PdG metabolites aligned by sample collection date. Figure 4 portrays the profile for the same sampling interval obtained from measurements of fecal steroid metabolites, similarly aligned.

Figures 5 and 6 represent an expanded window of a segment of the graphics depicted in Figures 3 and 4 that illustrates consecutive ovarian cycles in the same female prior to her second pregnancy obtained from urinary and fecal hormone measurements of steroids and their metabolites. Urinary (Fig. 5) and fecal (Fig. 6) estrogen and progesterone rise and fall almost concurrently at 14–16 day intervals, with progesterone metabolites leading estrogen metabolites in urine.

The urinary metabolites of the pre-adolescent female (animal 3) exhibited a flat profile (not shown) with estrone conjugate levels ranging between 10 and 30 ng/mg Cr and pregnanediol-3-glucuronide levels between < 0.01 and 0.02 μg/mg Cr. The fecal profile (not shown) for this animal was similar: estrone conjugate levels varied between 1.0 and 8.0 ng/g and 100 and 300 ng/g for pregnanediol-3-glucuronide.

The adolescent female (animal 5) exhibited a urinary profile (not shown) with a similar estrone conjugate baseline (10–40 ng/mg Cr) compared to the pre-adolescent female and a slightly more elevated PdG baseline (0.13–0.38 μg/mg Cr). In

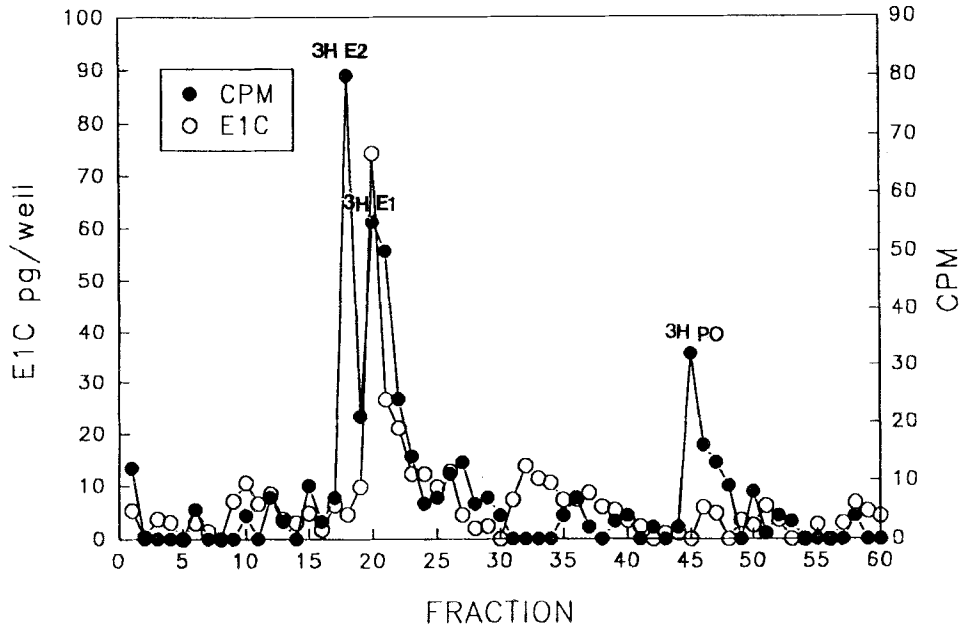

Fig. 2. Co-chromatography on a *Pithecia pithecia* fecal sample with high estrogen content as measured by E_1C EIA (> 10 μg/mg Cr). Tritiated estradiol, estrone, and progesterone (^3H E_2, ^3H E_1, and ^3H Po) are depicted by closed circles, and their units of measurement are on the right ordinate as counts/minute (CPM). Immunoreactive estrone conjugates are represented by open circles, and the units of measurement are in picograms/well (pg/well) on the left ordinate. The abscissa represents fractions collected on a 35% acetonitrile:65% dH_2O gradient column at a 1.0 ml/min flow rate and 0.5 ml fractions [Shideler et al., 1993, in press].

addition, the adolescent female exhibited a first cycle attempt two-thirds of the way through the present study interval, during which E_1C levels reached 525 ng/mg Cr, and PdG levels 0.75 μg/mg Cr, before they returned to baseline values where they remained for the rest of the study. Pre-adolescent baseline fecal estrogen and progesterone metabolite levels ranged between 1 and 8 ng/g for E_1C and 100 and 300 ng/g while adolescent E_1C levels ranged between 5 and 15 ng/g and PdG levels between 200 and 500 ng/g. First adolescent cycle peaks of 70 ng/g E_1C and 5,000 ng/g PdG were observed.

DISCUSSION

The high performance liquid chromatography separations and co-chromatography of saki fecal sample eluates yielded a broad immunoreactive progesterone metabolite peak that was both more polar than the tritiated progesterone peak and which overlapped it. The initial polarity of this peak and its overlap with the

Fig. 3. The urinary estrogen and progesterone metabolite excretion pattern of one *Pithecia pithecia* female spanning 632 days, aligned by sample collection date. The scale on the left ordinate is estrone conjugate levels in micrograms/milligram creatinine (μg/mg Creatinine; open circles); the right ordinate is pregnanediol-3-glucuronide levels in micrograms/milligram creatinine (μg/mg Creatinine; closed circles). The abscissa scale represents consecutive sample collection days. The first 140–160 days reflect pregnancy and parturition (female live birth) and the next 130–150 days, lactational amenorrhea. This is followed by approximately 100 days of repetitive ovarian cycles and 150–170 days representing conception, pregnancy, and parturition (male live birth).

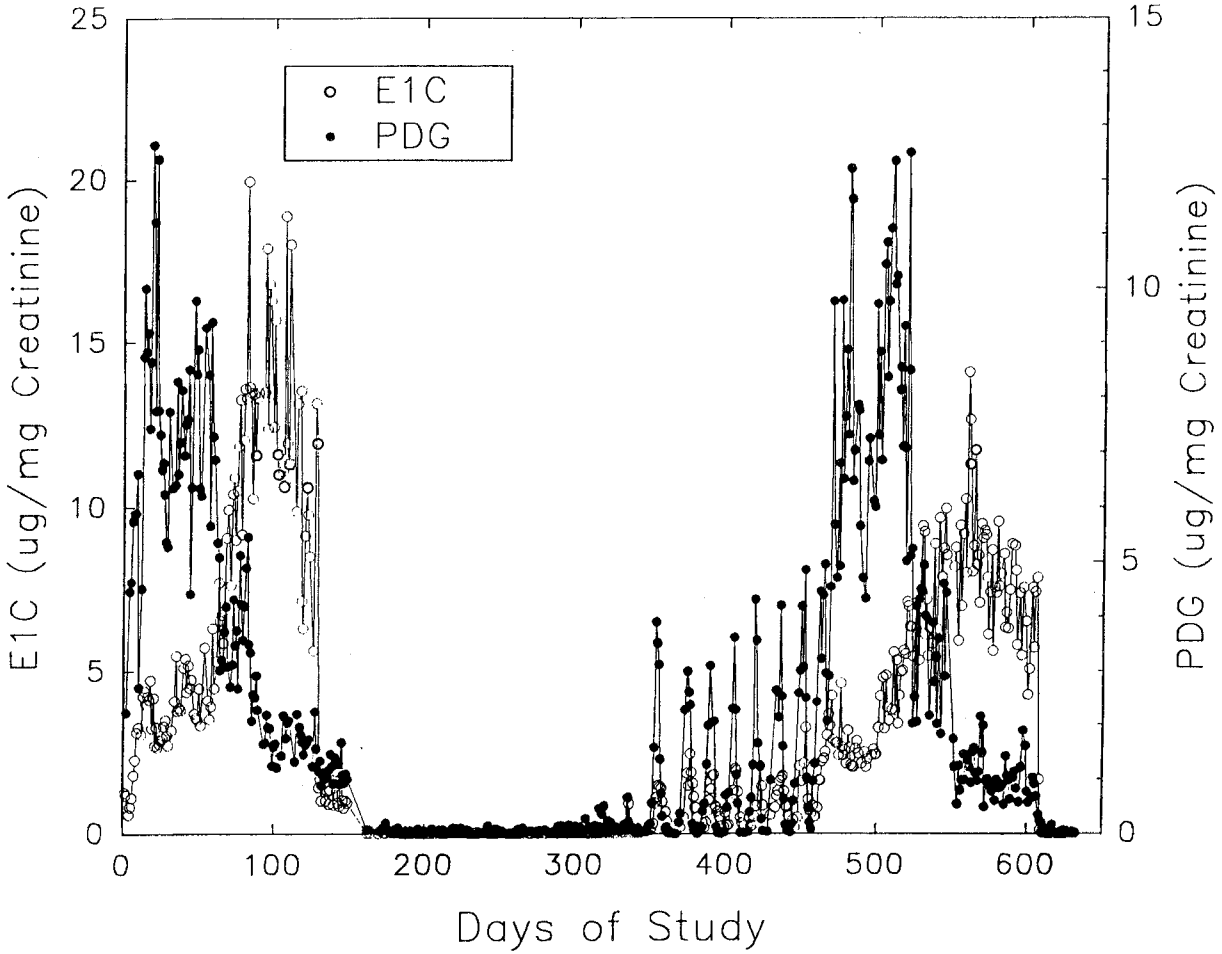
E1C
PDG
E1C (ug/mg Creatinine)
PDG (ug/mg Creatinine)
Days of Study
0
5
10
15
20
25
0
100
200
300
400
500
600

Fig. 3.

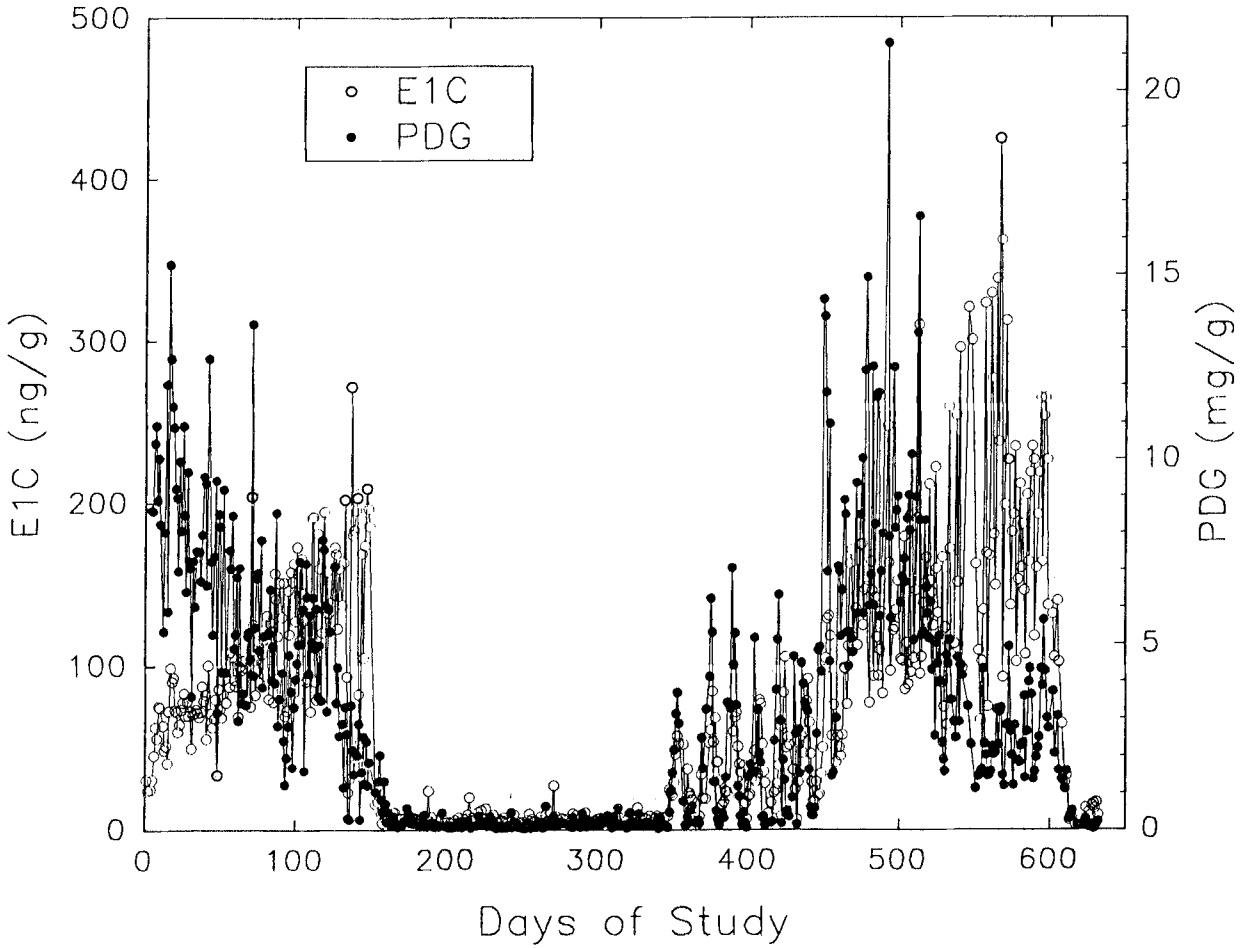
E1C
PDG
E1C (ng/g)
PDG (mg/g)
Days of Study
0
100
200
300
400
500
600
0
5
10
15
20

Fig. 4.

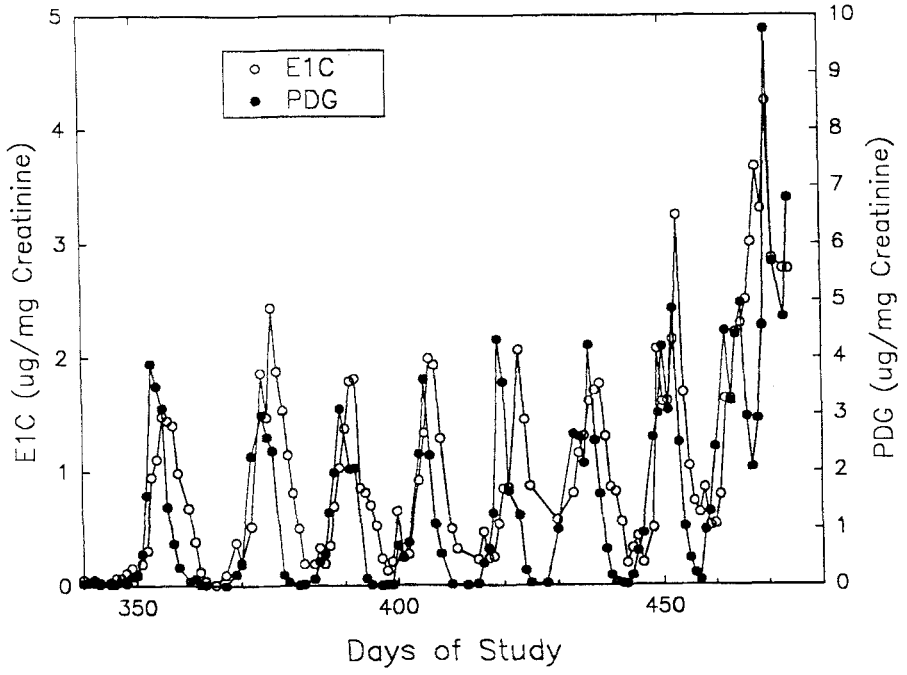

Fig. 5. An expanded window of a segment of the urinary estrogen and progesterone metabolite pattern depicted in Figure 3, representing repetitive ovarian cycles, aligned by sample collection date. The scale on the left ordinate is estrone conjugate levels in micrograms/milligram creatinine (μg/mg Creatinine; open circles); the right ordinate is pregnanediol-3-glucuronide levels in micrograms/milligram creatinine (μg/mg Creatinine; closed circles). The abscissa scale represents consecutive sample collection days.

tritiated progesterone peak was consistent with the elution pattern of PdG when using the same system in this laboratory. The observed immunoreactive estrogen peak was coincident with tritiated estrone.

Unconjugated estrone occurred in larger proportion than conjugated estrone in *Pithecia pithecia* fecal samples in adolescent, cycling, and pregnant phases of the reproductive cycle. The capacity of the estrone conjugate EIA antibody to detect both conjugated and unconjugated estrone was highly advantageous in assessing saki ovarian activity. The relative specificity of the polyclonal pregnanediol-3-glucuronide EIA antibody, on the other hand, was advantageous when applied to cycling and adolescent *Pithecia pithecia* since the largest proportion of progesterone metabolites measured by the PdG radioimmunoassay was conjugated, indicating that this antibody was a valid choice to apply to the measurement of progesterone metabolites in white-faced saki feces. While a greater proportion of unconjugated progesterone metabolites was present in pregnancy than conjugated, the ability to detect the pregnancy event was not diminished since the absolute increase in Po metabolites was so great. It is possible that this shift from predom-

Fig. 4. The fecal estrogen and progesterone metabolite excretion pattern of the same *Pithecia pithecia* female for the same sampling interval depicted in Fig. 3. The scale on the left ordinate is estrone conjugate levels in nanograms/gram wet weight feces (ng/g; open circles); the right ordinate is pregnanediol-3-glucuronide levels in milligrams/gram wet weight feces (mg/g; closed circles). See legend of Fig. 3 for more details.

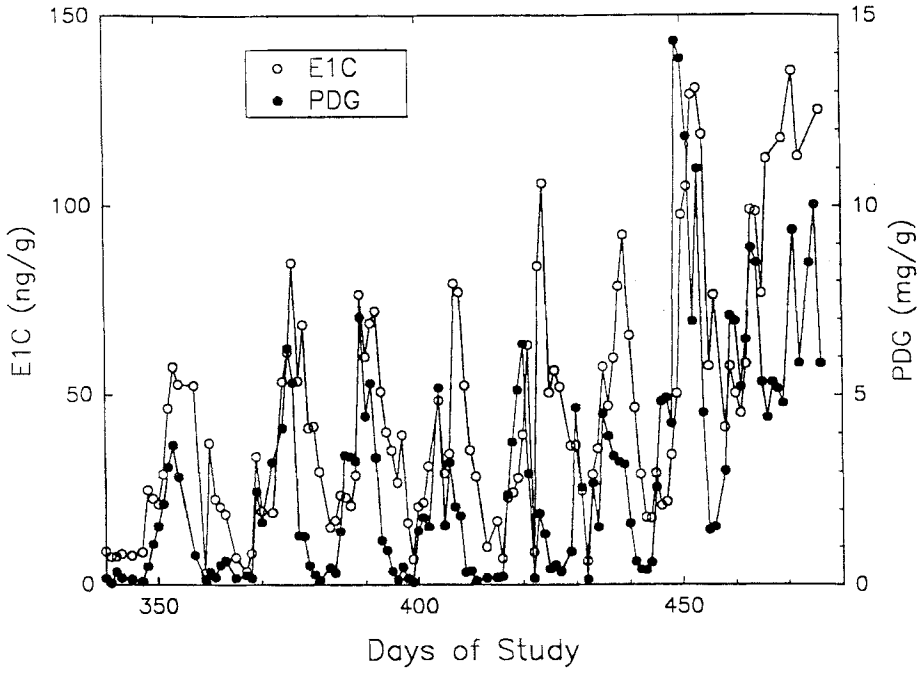

Fig. 6. An expanded window of a segment of the fecal estrogen and progesterone metabolite pattern depicted in Fig. 4, representing repetitive ovarian cycles, aligned by sample collection date, for comparison to Fig. 5. The scale on the left ordinate is estrone conjugate levels in nanograms/gram wet fecal weight (ng/g; open circles); the right ordinate, pregnanediol-3-glucuronide in milligrams/gram wet fecal wet (mg/g; closed circles). The abscissa scale represents consecutive sample collection dates.

inantly conjugated to unconjugated progesterone metabolites reflected the inability of metabolism to maintain conjugation when circulating concentrations were elevated.

The relationship between serum secretion and urinary excretion of steroid hormones has been documented in human and non-human primates [Monfort et al., 1987; Shideler et al., 1989, 1990; Lasley et al., 1991]. Urinary measurements have been found to reflect the same ovarian events as serum measurements. Furthermore, in addition to data from Risler et al. [1987] and Wasser et al. [1988, 1992a] on the relationship between blood and feces in *Macaca nemestrina* and *Papio cynocephalus cynocephalus,* respectively, Shideler et al. [1993] have presented a comprehensive evaluation of this relationship with daily serum, urinary, and fecal measurements for captive *Macaca fascicularis.* These studies agree that the changes in hormone concentrations observed in blood, urine, and feces reflect the same ovarian events. A lag time between hormone levels in excreta and serum has been reported to be approximately 1–2 days [Wasser et al., 1992a,b; Shideler et al., 1993, in press]. Similar descriptions of the relationship between fecal hormones and serum and urinary hormone metabolites have been reported by several researchers on many mammalian species: goats [Holtz, 1992], black-footed ferrets, Asian small-clawed otters, North American river otters, cheetah, maned wolves, tigers [Gross, 1992], marmosets, tamarins [Hodges & Heistermann, 1992], selected clawed neotropical primates [Pryce & Döbeli, 1992], and Asian elephants [Hoppen et al., 1992]—all of which found statistically significant relationships between serum and fecal hormone values.

Animals in the present study were not available for even opportunistic venipuncture; hence, the measurement of ovarian hormones in the urine and feces of *Pithecia pithecia* was undertaken on the assumption that urinary profiles reflected serum profiles based on previously demonstrated relationships between serum hormone concentrations and urinary hormone excretion. The correlation coefficients between urinary and fecal evaluation of estrogen and progesterone metabolites in *Pithecia pithecia* by the same assay systems were highly statistically significant in all cases but one animal (animal 3) which represented an immature, non-cycling female. Interestingly, another animal (animal 5) was also adolescent and reached menarche during the study. The correlations between the latter animal's fecal and urinary measurements were statistically significant while the former's were not.

Using a small amount of feces (0.25 g) for solubilization in 2.5 ml liquid produced relatively low recoveries. While recoveries can nearly be doubled by doubling both fecal mass and solubilizer volume and using larger extraction tubes, as determined by initial extraction studies in this laboratory, the larger proportions required different handling (i.e., were more labor intensive). Since we lost nothing in this species' profiles of interest to us by using the less cumbersome extraction method with lower efficiencies, we proceeded to use the easier laboratory method in the present study.

Like the findings of Wasser et al. [1992a], the expression of fecal steroid metabolites on a mass per (wet weight) feces basis was sufficient to provide strong and consistent fecal profiles in all mature individuals studied. The degree to which the relationship between hormone concentration and wet weight fecal mass is found to be constant for other captive species or for free-ranging animals with more varied diets and water intake remains to be determined. Wasser et al. [1992a,b] have already assessed the effects of fourfold increases of dietary fiber on excreted fecal steroids and demonstrated that drying feces was sufficient to control for most dietary effects and that cholestanone showed promise as an indexer of fecal excretion rate.

While other steroids or steroid metabolites like estradiol and progesterone are likely present in *Pithecia* excreta, the present initial study focused only on estrone and pregnanediol because these are known to be primary or major metabolites in other South American primates [Czekala et al., 1981; Hodges et al., 1979, 1981, 1983; Hodges & Eastman, 1984; Ziegler et al., 1989, 1990] and the enzyme immunoassays for these metabolites were immediately available. Furthermore, the comparison of fecal to urinary excretion required the use of assays that can detect the steroid metabolites common to both fecal and urinary compartments. Future investigations may demonstrate that other ovarian or placental steroid metabolites are more informative.

In this study, the amounts of steroid excreted in feces were comparable to that excreted in urine in terms of reflecting daily reproductive events in mature pregnant and nonpregnant females. The lack of a clear hormonal pattern in the young, immature animal and in post-partum females represented an equally reliable indicator of the absence of ovarian activity. Thus, the approach presented here has promise for monitoring all major reproductive events throughout the reproductive life of *Pithecia pithecia* and closely related species. Preliminary results from the application of this approach to non-primate mammalian species, such as elk, bison, and feral horses, indicate that this technique will work in these species as well [Kirkpatrick et al., 1992; Kirkpatrick, personal communication]. The application of the method presented here, or of any other method, however, should be tested to assess the validity of their use on an individual species basis.

CONCLUSIONS

1. The method presented in this report for extracting and measuring ovarian steroids in the feces of captive *Pithecia pithecia* is able to characterize major reproductive events in the reproductive life of females of this species.

2. The relationships between urine and fecal steroid excretion profiles for individual animals were found to be statistically significant in all but one immature animal, implying that fecal hormone excretion reflects circulating hormones, assuming a direct relationship between serum and urinary hormone profiles exits.

3. Progesterone metabolism and excretion (but not estrogen) were found to vary within animals in different reproductive conditions. This variation was observed during pregnancy but did not impact the ability of the PdG EIA to detect the pregnancy event.

ACKNOWLEDGMENTS

The use of the white-faced sakis in these studies was provided by the Roger Williams Park Zoo. Some of these data were presented at the XIVth Congress of the International Primatological Society, Strasbourg, France, August, 1992, and were summarized in abstracts published by the International Primate Society [Savage et al., 1992a,b].

The studies conducted were in compliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the Roger Williams Park Zoo. The authors thank the Roger Williams Park Zoo and their keeper staff for their contributions to the execution of these studies, as well as the volunteers and students from Brown University and the University of Rhode Island for sample collections. We also thank Pete Lohstroh and Francisco Morán for their excellent technical assistance in the laboratory and three reviewers whose comments contributed to making the manuscript more focused and clear. This study was funded by NSF BNS-8719698 (Shideler, Lasley), and IMS Conservation Project Support IC-12182-91 (Savage).

REFERENCES

Bamberg, E.; Möstel, E.; Patzl, M.; King, G.J. Pregnancy diagnosis by enzyme immunoassay of estrogens in feces from nondomestic species. JOURNAL OF ZOO AND WILDLIFE MEDICINE 22(1):73–77, 1991.

Czekala, N.M.; Hodges, J.K.; Lasley, B.L. Pregnancy monitoring in diverse primate species by estrogen and bioactive luteinizing hormone determinations in small volumes of urine. JOURNAL OF MEDICAL PRIMATOLOGY 10:1–15, 1981.

Desaulneirs, D.; Goff, A.K.; Betteridge, K.J.; Rowell, J.; Flood, P.F. Reproductive hormone concentrations in faeces during the oestrous cycle and pregnancy in cattle (*Bos taurus*) and muskoxen (*Bos moschatus*). CANADIAN JOURNAL OF ZOOLOGY 67:1148–1154, 1989.

Gross, T.S. Development and use of faecal steroid analyses in several carnivore species. Pp. 55–61 in THE FIRST INTERNATIONAL SYMPOSIUM ON FAECAL STEROID MONITORING IN ZOO ANIMALS. Proceedings. Utrecht, Belgium, Royal Rotterdam Zoological and Botanical Gardens, 1992.

Hodges, J.K.; Brand, H.; Henderson, C.; Kelly, R.W. Levels of circulating and urinary oestrogens during pregnancy in the marmoset monkey (*Callithrix jacchus*). JOURNAL OF REPRODUCTION AND FERTILITY 67:73–82, 1983.

Hodges, J.K.; Czekala, N.M.; Lasley, B.L. Estrogen and luteinizing hormone secretion in diverse primate species from simplified urinary analysis. JOURNAL OF MEDICAL PRIMATOLOGY 8:349–364, 1979.

Hodges, J.K.; Eastman, S.A.K. Monitoring ovarian function in marmosets and tamarins by the measurement of urinary estrogen metabolites. AMERICAN JOURNAL OF PRIMATOLOGY 6:187–197, 1984.

Hodges, J.K.; Gulick, B.A.; Czekala, N.M.; Lasley, B.L. Comparison of urinary oestro-

gen excretion in South American primates. JOURNAL OF REPRODUCTION AND FERTILITY 61:83–90, 1981.

Hodges, J.K.; Heistermann, M. Faecal steroid excretion during the ovarian cycle of marmosets and tamarins. Pp. 47–50 in THE FIRST INTERNATIONAL SYMPOSIUM ON FAECAL STEROID MONITORING IN ZOO ANIMALS. Proceedings. Utrecht, Belgium, Royal Rotterdam Zoological and Botanical Gardens, 1992.

Holt, W. Pregnancy diagnosis through faecal estrogens in goats. Pp. 10–15 in THE FIRST INTERNATIONAL SYMPOSIUM ON FAECAL STEROID MONITORING IN ZOO ANIMALS. Proceedings. Utrecht, Belgium, Royal Rotterdam Zoological and Botanical Gardens, 1992.

Hoppen, H.O.; Diaz de Aguirre, L.; Hagenbeck, M.; Boer, M.; Schwarzenberger, F. Progesterone metabolites in elephant feces. Pp. 51–54 in THE FIRST INTERNATIONAL SYMPOSIUM ON FAECAL STEROID MONITORING IN ZOO ANIMALS. Proceedings. Utrecht, Belgium, Royal Rotterdam Zoological and Botanical Gardens, 1992.

Kirkpatrick, J.F.; Shideler, S.E.; Lasley, B.L.; Turner, J.W. Pregnancy determination in uncaptured feral horses by means of fecal steroid conjugates. THERIOGENOLOGY 35:753–760, 1991.

Kirkpatrick, J.F.; Shideler, S.E.; Lasley, B.L., Turner, J.W., Jr.; Czekala, N.M. Field application of faecal steroid monitoring to free-ranging wild-life. Pp. 25–34 in THE FIRST INTERNATIONAL SYMPOSIUM ON FAECAL STEROID MONITORING IN ZOO ANIMALS. Proceedings. Utrecht, Belgium, Royal Rotterdam Zoological and Botanical Gardens, 1992.

Kirkpatrick, J.F.; Shideler, S.E.; Turner, J.W. Pregnancy determination in uncaptured feral horses based on steroid metabolites in urine-soaked snow and free steroids in feces. CANADIAN JOURNAL OF ZOOLOGY 68:2576–2579, 1990.

Lasley, B.L.; Kirkpatrick, J.F. Monitoring ovarian function in captive and free-ranging wildlife by means of urinary and fecal steroids. JOURNAL OF ZOO AND WILDLIFE MEDICINE 22(1):23–31, 1991.

Lasley, B.L.; Shideler, S.E.; Munro, C.J. A prototype for ovulation detection. AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY 165, 6(2):2003–2007, 1991.

Lasley, B.L.; Stabenfeldt, G.H.; Overstreet, J.W.; Hanson, F.W.; Czekala, N.M.; Munro, C.J. Urinary hormones at the time of ovulation and implantation. FERTILITY AND STERILITY 43:861–867, 1985.

Messier, F.; Desaulniers, D.M.; Goff, A.K.; Nault, R.; Patenaude, R.; Crête, M. Pregnancy diagnosis in caribou using progesterone and estrogen concentrations in feces. JOURNAL OF WILDLIFE MANAGEMENT, (in press).

Mitchell, W.R.; Presley, S.; Czekala, N.M.; Lasley, B.L. Urinary immunoreactive estrogen and pregnanediol-3-glucuronide during the normal menstrual cycle of the lowland gorilla (*Gorilla gorilla*). AMERICAN JOURNAL OF PRIMATOLOGY 2:167–175, 1983.

Monfort, S.L.; Hess, D.L.; Shideler, S.E.; Samuels, S.J.; Hendrickx, A.G.; Lasley, B.L. Comparison of serum estradiol to urinary estrone conjugates in the rhesus macaque (*Macaca mulatta*). BIOLOGY OF REPRODUCTION 37:832–837, 1987.

Munro, C.J.; Stabenfeldt, G.H.; Cragun, J.R.; Addiego, L.A.; Overstreet, J.W.; Lasley, B.L. Relationship of serum estradiol and progesterone concentrations to the excretion profiles of their major urinary metabolites as measured by enzyme immunoassay and radioimmunoassay. JOURNAL OF CLINICAL CHEMISTRY 37:838–844, 1991.

Pryce, C.R,; Döbeli, M. Monitoring oestrogen excretion in neotropical monkeys: Coping with different metabolic strategies. Pp. 35–46 in THE FIRST INTERNATIONAL SYMPOSIUM ON FAECAL STEROID MONITORING IN ZOO ANIMALS. Proceedings. Utrecht, Belgium, Royal Rotterdam Zoological and Botanical Gardens, 1992.

Risler, L.; Wasser, S.K.; Sackett, G.P. Measurement of excreted steroids in *Macaca nemestrina*. AMERICAN JOURNAL OF PRIMATOLOGY 12:91–100, 1987.

Royal Rotterdam Zoological and Botanical Gardens. THE FIRST INTERNATIONAL SYMPOSIUM ON FAECAL STEROID MONITORING IN ZOO ANIMALS. Proceedings. Utrecht, Belgium, 1992.

Savage, A.; Lasley, B.L., Shideler, S.E. Studying reproductive functioning in zoo animals: A case study of the white-faced saki program at the Roger Williams Park Zoo. P. 203 (abstract 392) in XIVTH CONGRESS OF THE INTERNATIONAL PRIMATOLOGICAL SOCIETY. August, 1992b.

Savage, A.; Shideler, S.E.; Moorman, E.A.; Ortuno, A.; Whittier, K.K.; McKinney, J. The reproductive biology of the white-faced saki (*Pithecia pithecia*) in captivity. P 59 (abstract 98) in XIVTH CONGRESS OF THE INTERNATIONAL PRIMATOLOGICAL SOCIETY. August, 1992a.

Shideler, S.E.; Czekala, N.M.; Kasman, L.H.; Lindburg, D.G.; Lasley, B.L. Monitoring ovulation and implantation in the lion-

tailed macaque (*Macaca silenus*) through urinary estrone conjugate evaluations. BIOLOGY OF REPRODUCTION 29:905–911, 1983.

Shideler, S.E.; DeVane, G.W.; Kalra, P.S.; Benirschke, K.; Lasley, B.L. Ovarian-pituitary hormone interactions during the perimenopause. MATURITAS 11:331–339, 1989.

Shideler, S.E.; Munro, C.J.; Laughlin, L.; Tell, L.; Owiti, G.; Lasley, B.L. The relationship of serum estradiol and progesterone concentrations to the enzyme immunoassay measurements of urinary estrone conjugates and immunoreactive pregnanediol-3-glucuronide in *Macaca mulatta*. AMERICAN JOURNAL OF PRIMATOLOGY 22:113–122, 1990.

Shideler, S.E.; Ortuño, A.M.; Moràn, F.M.; Moorman, E.A.; Lasley, B.L. Simple extraction and enzyme immunoassays for estrogen and progesterone metabolites in the feces of *Macaca fascicularis* during nonconceptive and conceptive ovarian cycles. BIOLOGY OF REPRODUCTION 48: 1290–1298, 1993.

Shideler, S.E.; Shackleton, C.H.L.; Moràn, F.M.; Stauffer, P.; Lohstroh, P.N.; Lasley, B.L. Enzyme immunoassays for ovarian steroid metabolites in the urine of *Macaca fascicularis*. JOURNAL OF MEDICAL PRIMATOLOGY 22 (in press).

Taussky, H.H. A microcolormetric determination of creatine in urine by the Jaffe reaction. JOURNAL OF BIOLOGICAL CHEMISTRY 208:853–861, 1954.

Wasser, S.K.; Monfort, S.L.; Wildt, D.E. Rapid extraction of faecal steroids for measuring reproductive cyclicity and early pregnancy in free-ranging yellow baboons (*Papio cynocephalus cynocephalus*). JOURNAL OF REPRODUCTION AND FERTILITY 92:415–423, 1991.

Wasser, S.K.; Risler, L.; Steiner, R.A. Excreted steroids in primate feces over the menstrual cycle and pregnancy. BIOLOGY OF REPRODUCTION 39:862–872, 1988.

Wasser, S.K.; Risler, L.; Wasser, L.M. Use of techniques to extract steroid hormones from primate feces. PRIMATE REPORTS 14:194–195 (abstract), 1986.

Wasser, S.K.; Thomas, R.I.; Nair, P.P.; Monfort, S.L. Controlling for effects of diet on excretion of fecal progestogens. BIOLOGY OF REPRODUCTION 46(suppl 1):97 (abstract 188), 1992b.

Wasser, S.K.; Thomas, R.I.; Nair, P.P.; Monfort, S.L.; Wildt, D.E. Effect of diet on fecal steroid measurements. Pp. 10–15 in THE FIRST INTERNATIONAL SYMPOSIUM ON FAECAL STEROID MONITORING IN ZOO ANIMALS. Proceedings. Utrecht, Belgium, Royal Rotterdam Zoological and Botanical Gardens, 1992a.

Ziegler, T.E.; Bridson, W.E.; Snowdon, C.T.; Eman, S. Urinary gonadotropin and estrogen excretion during the postpartum estrus, conception, and pregnancy in the cotton-top tamarin (*Saguinus oedipus oedipus*). AMERICAN JOURNAL OF PRIMATOLOGY 12:127–140, 1987.

Ziegler, T.E.; Sholl, S.A.; Scheffler, G.; Haggerty, M.A.; Lasley, B.L. Excretion of estrone, estradiol, and progesterone in the urine and feces of the female cotton-top tamarin (*Saguinus oedipus oedipus*). AMERICAN JOURNAL OF PRIMATOLOGY 17:185–195, 1989.

Ziegler, T.E.; Snowdon, C.T.; Bridson, W.E. Reproductive performance and excretion of urinary estrogens and gonadotropins in the female pygmy marmoset (*Cebuella pygmaea*). AMERICAN JOURNAL OF PRIMATOLOGY 22:191–203, 1990.