The First International Symposium on Faecal Steroid Monitoring in Zoo Animals

Proceedings

February 28 and 29, 1992

Royal Rotterdam Zoological and Botanical Gardens

The First International Symposium on Faecal Steroid Monitoring in Zoo Animals

February 28 and 29, 1992
Royal Rotterdam Zoological and Botanical Gardens

Organization: Willem Schaftenaar, Veterinarian Rotterdam Zoo
Robert M. Buiter, Biological/Veterinary Department Rotterdam Zoo
Steph J. Dieleman, Faculty of Veterinary Medicine, Utrecht University

Field Application of Faecal Steroid Monitoring to Free-Ranging Wildlife

Jay F. Kirkpatrick[1], S. E. Shideler[2], B. L. Lasley[2], J. W. Turner, Jr.[3], and N. M. Czekala[4]

[1]Deaconess Research Institute, 1500 Poly, Billings, MT 59102, USA;
[2]Institute for Toxicology and Environmental Health Research, University of California, Davis, CA 95616, USA;
[3]Department of Physiology, Medical College of Ohio, Toledo, OH 43699, USA;
[4]Center for Reproduction of Endangered Species, San Diego Zoo, San Diego, CA 92112, USA.

Abstract

Reproductive function has been monitored by means of urinary or faecal steroid analysis in a large variety of captive exotic animals for a number of years, but application to true free-roaming wild species is a relatively recent event. Pregnancy diagnosis in feral horses (E. caballus) has been accomplished through the measurement of faecal oestrogens and steroid conjugates in order to study mechanisms of compensatory reproduction, rates of fetal loss, the efficacy of immuno contraception, and the possibility of induced abortion by new herd stallions. More recently, faecal free progesterone, oestrone conjugates and non-specific immunoreactive pregnanediol-3-glucuronide-like metabolites have been measured in the mare in order to monitor ovulation and the oestrus cycle. Among free-roaming North American bison (Bison bison), faecal progesterone and oestrogens have been used to successfully diagnose pregnancy and faecal progesterone has been used to monitor the length of the oestrous cycle, and to detect ovulation in order to study the mechanisms of reproductive self-regulation. The use of faecal steroid analysis to monitor reproductive events can provide safe, accurate, and non-stressful methodologies for understanding reproduction in free-roaming wildlife.

Introduction

The effective management of free-roaming wildlife populations requires an understanding of the biology of each species and in particular, their reproductive physiology. Of the more than 4000 mammalian species, the complete reproductive endocrine profile has been characterized for fewer than 50 species (Lasley and Kirkpatrick 1991). Consequently, we have little knowledge of the reproductive biology of most wildlife in their native habitats. These gaps in our knowledge have left us with a poor understanding of the mammalian reproductive response to environmental cues, stress, population density changes, and genetic shifts in populations.

Until recently, the measurement of important parameters of reproductive biology in free-roaming wildlife required capture and the assessment of reproductive hormones circulating in the vascular bed. The live capture of free-roaming wildlife for purposes of reproductive studies traditionally required immobilization and restraint, events associated with enormous stress and sometimes significant mortality (Valkenburg et al. 1983; Seal et al. 1985), disruption of reproductive events (Ballard and Tobey 1981; Larsen and Gauthier 1989), and/or confounded endocrine status (Kirkpatrick et al. 1979).

An alternative approach to the study of ovarian function in free-roaming wildlife is based on the measurement of urinary and faecal steroids or their metabolites. The technology which permits remote monitoring of reproductive endocrinology was originally developed to evaluate the reproductive status of captive exotic mammals (Loskutoff et al. 1983; Lasley 1985) and its application has now been extended to free-roaming wildlife. The evaluation of faecal reproductive hormones in wildlife was preceded by urinary steroid metabolite analysis (Kirkpatrick et al. 1988, 1990a, 1990b,1991a,1992) and allowed

pregnancy determination, ovulation detection, and characterization of the estrous cycle in feral horses and bison. The evaluation of urinary steroid metabolites in free-roaming wildlife provides valuable information, but urine collection can be time-consuming and in some cases dangerous. The use of faecal steroid evaluation has proven successful with a number of domestic and captive exotic species (Schwarzenberger et al. 1990; Bamberg and Schwarzenberger 1990) and can provide the field biologist with an alternative and less labor- intensive strategy for monitoring ovarian reproductive events.

STUDIES IN FREE-ROAMING FERAL HORSES

In order to diagnose pregnancy among a population of feral horses which were being immunized against porcine zonae pellucidae, matched urine and fecal samples were collected from 34 uncaptured feral mares inhabiting Assateague Island National Seashore (MD). Urine samples collected directly from the ground were measured for estrone conjugates (E1C) and non-specific progesterone metabolites (iPdG) and compared with faecal total estrogen concentrations. Urinary E1C, which includes both the sulfate and glucuronide conjugates, were measured by enzyme immunoassay (EIA) as described by Shideler et al. (1990) and Kirkpatrick et al. (1990a), respectively. The total faecal estrogens were measured by radioimmunoassay as described by Kirkpatrick et al. (1990b). The mean faecal total estrogens for the 28 non-pregnant mares was 0.552 ± 0.08 ng/g faeces, and differed significantly from a mean value of 3.18 ± 0.70 ng/g for 5 pregnant mares. A comparison of urinary E1C and faecal total estrogens for 24 other mares is illustrated in Figure 1. The coefficient correlation between urinary E1C and faecal total estrogens was r = 0.928.

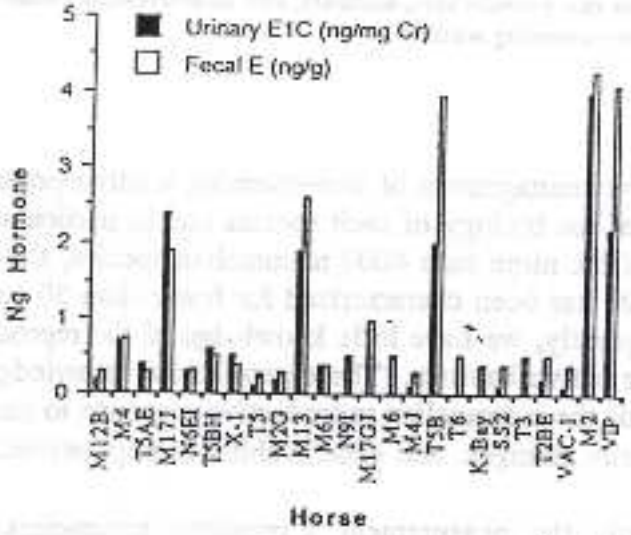

Figure 1. Comparison of urinary estrone conjugates (E1C) and faecal total estrogens (E) among 24 feral mares inhabiting Assateague Island National Seashore.

Although the measurement of faecal total estrogens in feral mares was accurate, organic extractions with ethyl acetate/hexane and the estrogen radioimmunoassay were time consuming and relatively expensive. The next experiment with feral horses focused upon the measurement of faecal steroid conjugates. The majority of conjugated steroid metabolites in mares is passed from the liver into the gut, where they are, in large measure, resorbed back into the vascular bed, transported to the kidney and excreted in the urine. This, of course, is the basis for the urinary steroid conjugate analysis. We reasoned that a smaller proportion of the conjugated steroids would be trapped in the gut and excreted in the faeces and that if they could be preserved as conjugates before bacterial degradation took place, they might be measured by the non-isotopic enzyme immunoassays used for urinary analysis. This strategy was applied to feral mares

successfully and the evaluation of simple water extracts of E1C and non-specific progesterone metabolites (iPdG) unique to the Equidae (Kirkpatrick et al. 1990c) yielded accurate predictors of pregnancy (Kirkpatrick et al. 1991b). Faecal E1C and iPdG concentrations for seven pregnant mares were 4.2 ± 0.8 ng/g and 1,411 ± 569.6 ng/g feces respectively. Faecal E1C and iPdG concentrations for 33 nonpregnant mares were 0.5 ± 0.1 and 32.8 ± 4.5 ng/g faeces, respectively. High Performance liquid Chromatography (HPLC) confirmed that E1C and iPdG were present in the faeces (see Figure 2).

The ovarian endocrine changes associated with the estrous cycle are dynamic and require frequent sampling in order to visualize the sequential events and the same non-capture approaches used for pregnancy detection can be used for monitoring cyclic ovarian function in free-ranging wildlife.

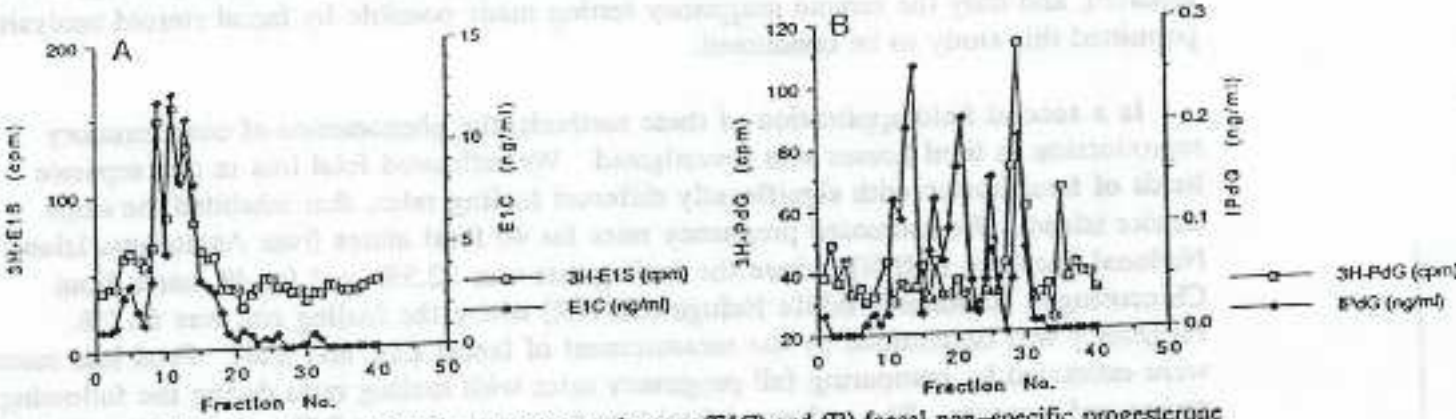

Figure 2. HPLC profiles of (A) faecal estrone conjugates (E1C) and (B) faecal non-specific progesterone metabolites (iPdG) in feral mares.

The estrous cycles of four mares, over a two month period were characterized by means of urinary E1C and iPdG and compared to faecal E1C, iPdG and progesterone. The faecal E1C and iPdG were measured by EIA as described above, and the progesterone was measured by RIA as described by Desaulniers et al. (1989). Correlation coefficients for urinary steroid conjugates versus faecal steroid conjugates or progesterone ranged from r = 0 to r = 0.83, but faecal E1C, iPdG, and progesterone all paralleled the qualitative changes in ovarian function assessed by urinary steroid conjugate analysis (see Figure 3). Faecal progesterone provided a more accurate assessment of the luteal phase than faecal iPdG.

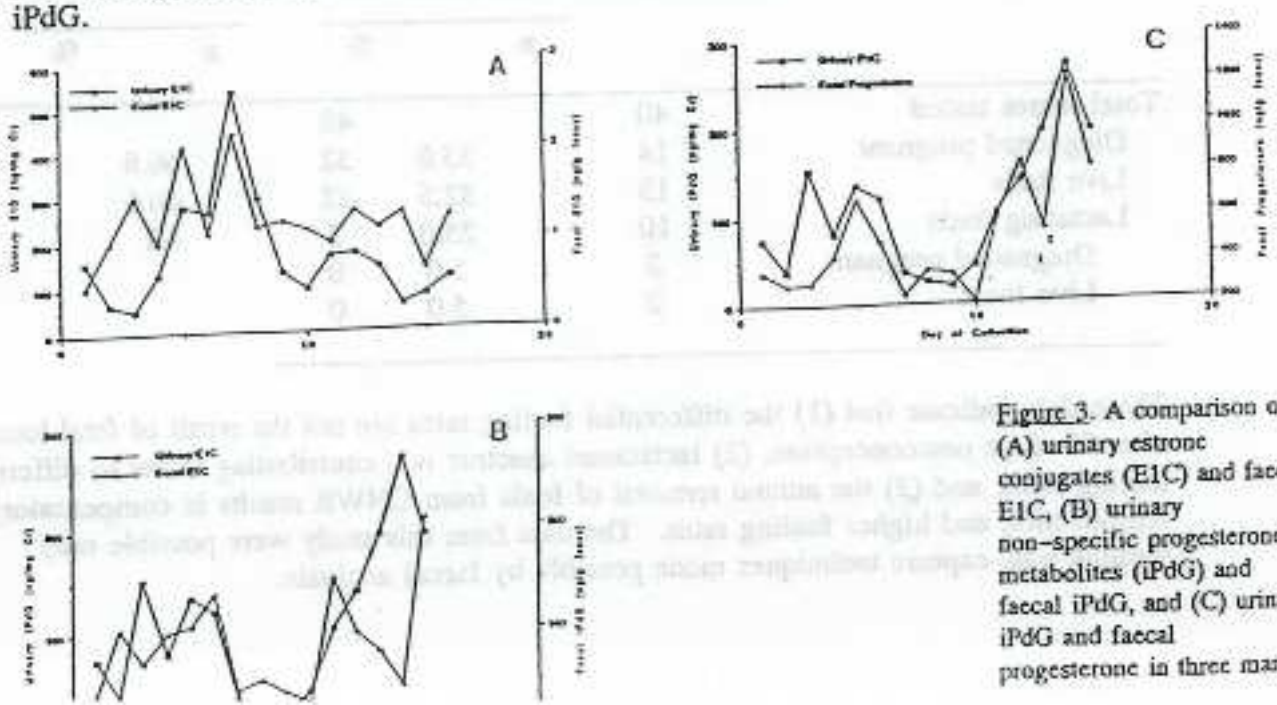

Figure 3. A comparison of (A) urinary estrone conjugates (E1C) and faecal E1C, (B) urinary non-specific progesterone metabolites (iPdG) and faecal iPdG, and (C) urinary iPdG and faecal progesterone in three mares.

These methods were next applied to two separate questions regarding the biology of the feral horse. In the first study, the phenomenon of forced copulation and induced abortion was investigated in a herd of feral horses inhabiting a coastal barrier island (Kirkpatrick and Turner 1991). Eight mares were diagnosed pregnant in August and October 1989 by means of fecal steroid metabolites, prior to documented changes in herd stallions. These mares were observed for harassment and forced copulation by the new stallions and for the presence of foals during the spring and summer of 1990. No incidents of harassment or attempts at forced copulation were witnessed and seven of the eight mares produced foals in 1990. These data indicate that forced copulation and induced abortion are not common events among all feral horse herds, as had been earlier reported, and only the remote pregnancy testing made possible by faecal steroid analysis permitted this study to be conducted.

In a second field application of these methods, the phenomenon of compensatory reproduction in feral horses was investigated. We estimated fetal loss in two separate herds of feral horses with significantly different foaling rates, that inhabited the same barrier island. We estimated pregnancy rates for 40 feral mares from Assateague Island National Seashore (AINS), where the foaling rate was 32.5% and for 48 mares from Chincoteague National Wildlife Refuge (CNWR) where the foaling rate was 62.5%. Pregnancy was determined by the measurement of faecal E1C and iPdG. Fetal loss rates were estimated by comparing fall pregnancy rates with foaling rates during the following spring and summer. The estimated fetal loss for mares from AINS and CNWR was 7.1% and 6.2%, respectively. Ten of the 40 mares from AINS (25%) were lactating and only 2 produced foals, whereas 2 of the 48 mares from CNWR (4.1%) were lactating and neither produced foals (see Table 1) (Kirkpatrick and Turner 1991d).

Table 1. Pregnancy and foaling rates (%) for lactating and non-lactating feral mares in 2 separate herds.

	Assateague Island National Seashore		Chincoteague National Wildlife Refuge	
	n	%	n	%
Total mares tested	40		48	
Diagnosed pregnant	14	35.0	32	66.6
Live foals	13	32.5	32	66.6
Lactating foals	10	25.0	2	4.1
Diagnosed pregnant	2	5.0	0	
Live foals	2	5.0	0	

These data indicate that (1) the differential foaling rates are not the result of fetal loss after 90-days postconception, (2) lactational anestrus is a contributing factor to differential foaling rates, and (3) the annual removal of foals from CNWR results in compensatory reproduction and higher foaling rates. The data from this study were possible only through non-capture techniques made possible by faecal analysis.

STUDIES IN FREE-ROAMING BISON

The North American bison (Bison bison) is a large intractable animal which poses a significant hazard to investigators. It is probably for this reason that the bison estrous cycle was not characterized endocrinologically until recently. This was first accomplished by means of urinary pregnanediol-3-‖-glucuronide (PdG) (Kirkpatrick et al. 1991a), but the hazards of even collecting urine from free-roaming bison are not trivial. Following the same line of reasoning that led to faecal analysis in feral horses, reproductive studies in bison also turned to faecal analysis. The accuracy of pregnancy diagnosis was first determined among 18 bison cows at approximately the third month of gestation, by means of urinary E1C and PdG and faecal total estrogens. Extrapolation from a previously studied species to a related wildlife species is often a profitable shortcut to the identification of a useful steroid or its metabolite. Thus, the rationale for measuring total estrogens was based on the work of Mostl et al. (1984) and Safer-Hermann et al.(1987), in which faecal 17-α-estradiol was predictive of pregnancy in domestic cows (Bos taurus) and red buffalo (Syncerus caffer nanus). Total estrogens were measured with an RIA utilizing an antibody (OP-11, Pantex Corporation, Santa Monica, CA) which had 30% cross-reactivity with 17-α-estradiol. Based on calving rates the next spring, the measurement of faecal total estrogens was 100% accurate when measured 3 months postconception (see Table 2).

In a second experiment, faecal samples were collected from 6 pregnant bison approximately 1, 3, and 5 months post-conception and total estrogens and progesterone was determined. The measurement of faecal progesterone was based on the work of Desaulniers et al. (1989), in which faecal progesterone was measured in domestic cows. Neither faecal estrogens nor progesterone were significantly elevated at 1 month post-conception. By 3 months post-conception, both steroids were significantly elevated and by 5 months post-conception pregnancy was even more discernable (see Figures 4A and 4B).

Table 2. Urinary PdG, E1C, and faecal total estrogen concentrations for pregnant and non-pregnant bison at approximately three months gestation.

Bison number	Urinary pregnanediol-glucuronide (ng/mg Cr[a])	Urinary estrone conjugates (ng/mg Cr)	Total fecal estrogens (ng/g feces)
G114	67.3	51.7	1.94
Y408	54.7	12.6	1.78
Y304	105.6	16.4	1.60
B62	87.2	9.4	1.40
B12	302.9	87.5	1.61
R3	156.3	23.6	1.47
B182	97.1	19.5	1.42
Y535	69.4	11.7	1.27
G404	76.8	43.3	1.44
R13	60.7	17.9	1.90
R512	120.7	14.8	1.73
G120	216.3	21.0	1.09
RX9	62.5	17.9	1.51
R513	68.5	27.1	1.39
RX7[b]	9.0	3.1	0.19
Y302[b]	3.6	5.2	0.15
Y432[b]	4.8	2.7	0.20
B200[b]	9.9	11.5	0.12

[a]Cr=creatinine
[b]Cows diagnosed as non-pregnant by rectal palpation and which did not deliver calves.

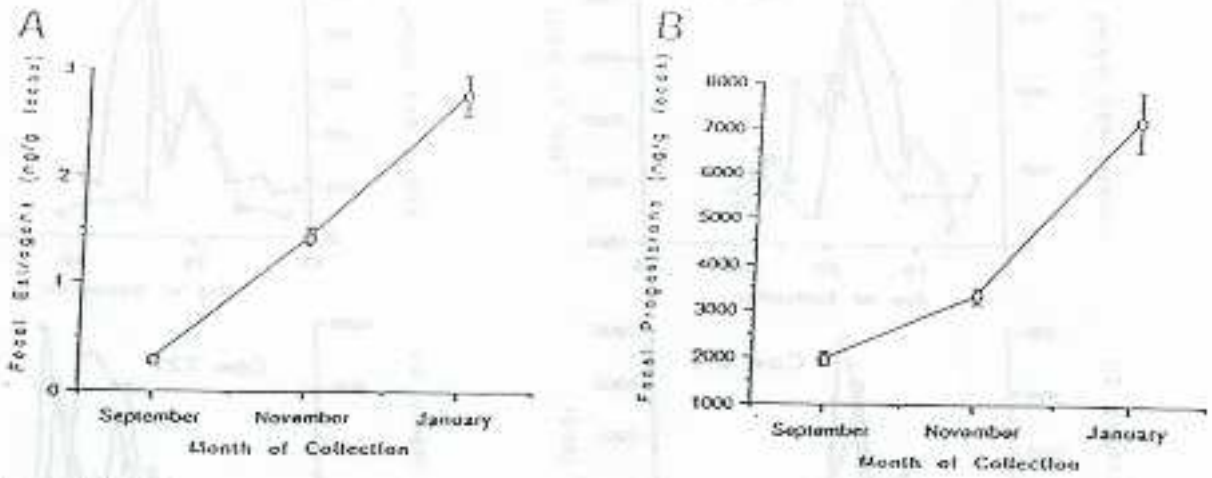

Figure 4. Faecal estrogens (A) and faecal progesterone (B) in 6 pregnant bison during the first five months post-conception.

In an attempt to detect ovulation in bison cows, urine and faecal samples were collected approximately every 2–4 days during the rutting season, between July 31 and August 31, from five sexually mature cow bison. Urine samples were analyzed for PdG by EIA as previously described (Kirkpatrick et al. 1991a). On some days both urine and fecal samples were collected from the same cow but on other days only a urine or faecal sample was collected. Faecal samples were weighed and extracted with diethyl ether and ry PdG and faecal progesterone concentrations during the 32-day collection period revealed a pattern consistent with ovulation and a non-conceptive luteal phase progesterone elevation and decline which is similar to the estrous cycle of the domestic cow (Asdell 1964), the European bison (Bison bonasus) (Krasinski and Raczynski 1967), and the North American bison (Kirkpatrick et al. 1991a). Four of the five bison demonstrated classic estrous behavior which was coincidental with both PdG and progesterone nadirs immediately preceeding luteal phase elevation. The correlation coefficient between urinary PdG and faecal progesterone for the five cows ranged from a low of $r = 0.31$ to a high of $r = 0.92$, however, in all five cases the basic luteal phase pattern of faecal progesterone excretion was evident. Figure 5 presents the luteal phase PdG and progesterone patterns for four cows.

Pregnancy and ovulation detection by means of faecal steroid analysis were next applied to the bison of Yellowstone National Park (YNP), in an attempt to elucidate the mechanisms of reproductive self-regulation and the reproductive effects of alledged infection with Brucella abortus. The first set of experiments was designed to test the validity of using faecal steroid analysis in free-ranging ungulates by measuring the correlation between observed reproductively-related behaviors and phenomena, and the expected endocrine patterns.

Between July 15 and August 31, 1990, 228 sexually mature bison cows were observed in the Hayden Valley of YNP. Of these 34.6% had calves at their sides. During this same period of time, 54 of these cows were observed being tended by a bull. Of these 54, 10 (18.5%) had calves at their sides and 44 (81.4%) did not, suggesting that ovulation was occurring predominantly among non-lactating cows. The percent of tended lactating and non-lactating cows, at two week intervals between July 15 and August 31, is shown in Fig.6.

During the same period of time faecal samples were collected from 121 cows which were not being tended at the time of collection. Of these 121 cows, 62 were lactating and 9 (14.5%) had ovulated, on the basis of faecal progesterone concentrations $\geq$ 3000 ng/g dry faeces; 59 were without calves and 25 (42.3%) had ovulated. The percent of ovulating lactating and non-lactating cows is shown in Figure 7. These data indicate that ovulation occurs in non-lactating cows at greater than twice the rate than in lactating

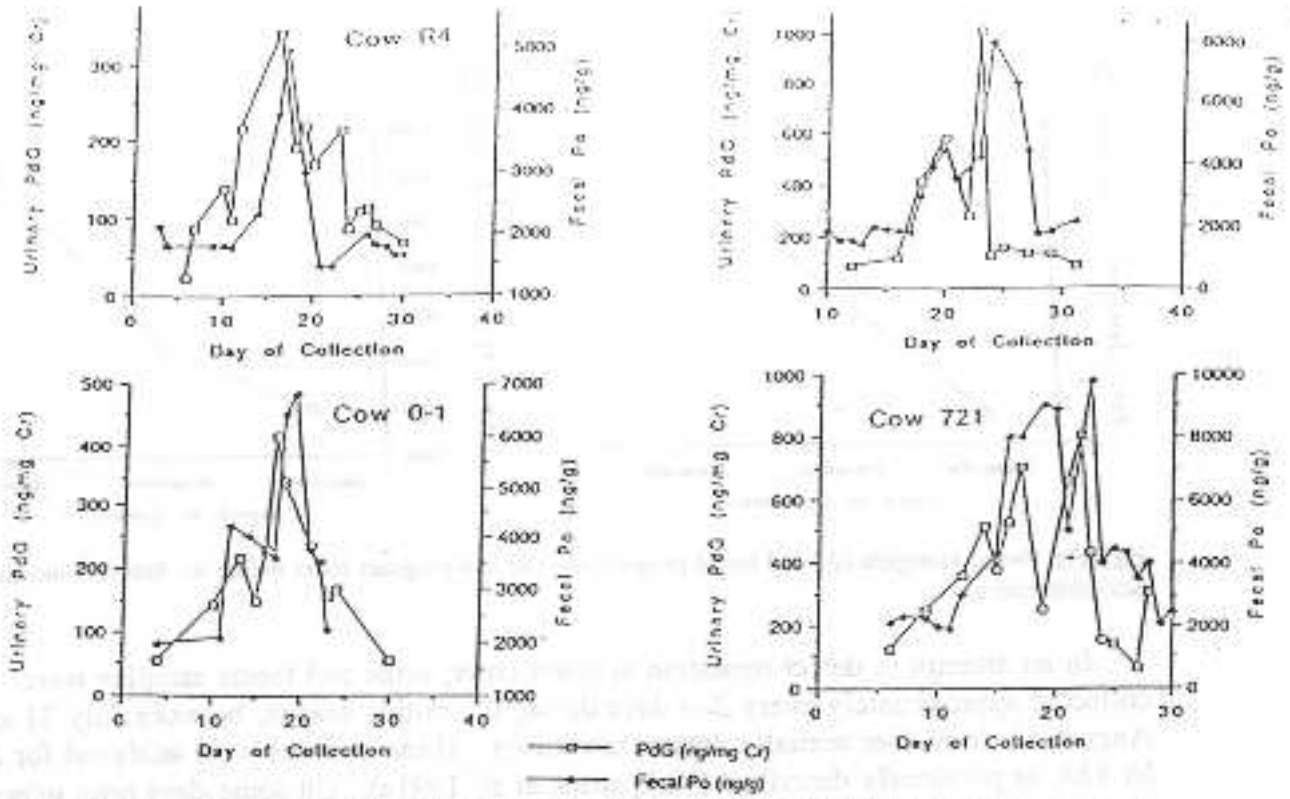

Figure 5. The estrous cycles of four North American bison cows characterized by urinary pregnanediol–3–glucuronide (PdG) and compared with faecal progesterone concentrations. Arrows indicate observed behavioral estrus.

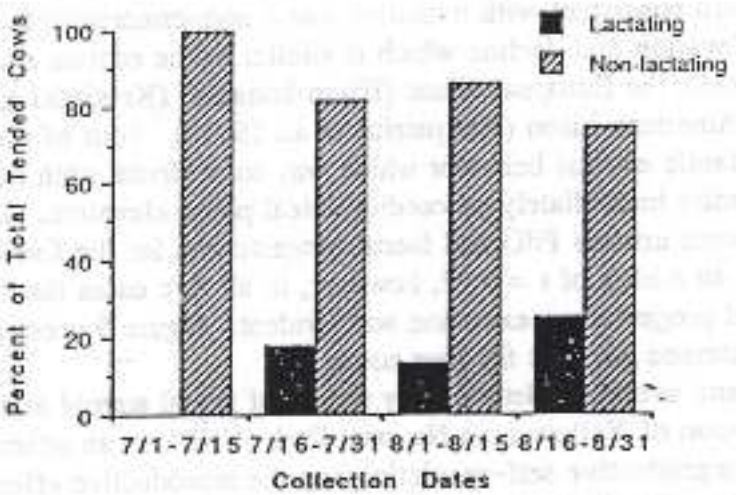

Figure 6. The percent of observed tended lactating and non–lactating bison cows among the Mary Mountain (Yellowstone) bison.

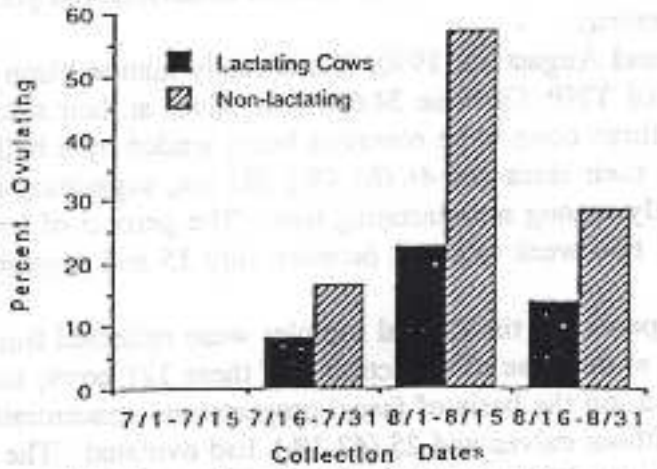

Figure 7. The percent of lactating and non–lactating Yellowstone bison cows ovulating during the rutting season. Ovulation was based on faecal progesterone concentration > 3,000 ng/g dry feces.

Twelve cows observed being tended by bulls were identified by individual physical characteristics and faecal samples were collected 3 – 5 days following the witnessed tending. Samples were analyzed for faecal progesterone in order to determine if witnessed tending and estrous behavior were reliable indicators of ovulation. Ten of the twelve cows had elevated faecal progesterone (≥ 3,000 ng/g). These data demonstrate that witnessed tending is a reliable indicator of ovulation.

Based on observed rates of tending of lactating and non–lactating cows, and the corresponding ovulation rates among these two groups, it would be expected that pregnancy rates are significantly higher among non–lactating cows. A total of 85 cows from the same herd was tested for pregnancy by means of faecal total estrogens and progesterone between late October and November 15, 1990. Of these 85 cows, 32 (37.6%) were pregnant. Of the total 32 pregnancies, 5 (15.6%) were among lactating cows and 27 (84.3%) were among non–lactating cows, as predicted by observed tending and ovulation rates during the previous rutting season. Collectively, over three years, the pregnancy rate for 255 cows from both the Hayden Valley and the Lamar Valley bison of YNP was 48.2 %, with 15.4% of the pregnancies among lactating cows. The pregnancy rates for each subpopulation and lactating and non–lactating cows is summarized in Table 3.

Table 3. Pregnancy rates for lactating bison cows in Yellowstone National Park's Northern Range herd, over three years.

Year	Subpopulation	No. Cows	No. Pregnant(%)	No. Pregnant Lactating Cows (%)
1989	NR	87	50 (57.4)	8 (16)
1990	NR	83	41 (49.3)	6 (14.6)
1991	MM	85	32 (37.6)	5 (15.6)

The remote monitoring of ovulation and pregnancy among bison of Yellowstone National Park by means of faecal steroids indicates that (1) as a rule, pregnancy occurs on alternate years, (2) approximately 15% of lactating cows will become pregnant on subsequent years, (3) the cause of low pregnancy rates in lactating cows is failure to ovulate, and (4) endocrine evidence of ovulation and pregnancy is consistent with observed reproductive behaviors.

Free–roaming wildlife species can provide a broad spectrum of valuable information with which to enhance our understanding of comparative reproduction and the reproductive strategies animals assume in natural settings. The use of faecal steroid analysis to monitor reproductive events can provide safe, accurate, and non–stressful methodologies for these types of studies. Additional research will soon provide even newer methods, such as aqueous extraction procedures, field pregnancy tests, and assays for other steroid metabolites. The application of this technology will depend upon the willingness to use it by scientists and wildlife managers.

ACKNOWLEDGEMENTS

The authors acknowledge the significant effort put forth on behalf of these studies by the following individuals: R. Keiper, B. Rogers, G. Olson, A. Rutberg, R. Naugle, K. Asis, D. Gudermuth, J. McCarthy, R. Flagan, J. Varley, H. Thomas, V. Kincy, K. Bancroft, and S. Broadbent. These studies were funded by National Park Service grant CA–1600–3–0005 (Kirkpatrick), National Science Foundation grants BNS–8719698–01 (Shideler) and DCB–8922800 (Kirkpatrick), NIEHS grant P42ESO4699 (Lasley), the World Society for the Protection of Animals, The Humane Society of the United States, the Associated Students of Eastern Montana College, and Sigma Xi.

Asdell, S. A. 1964. Patterns of Mammalian Reproduction, pp. 585–586, 606. Cornell University Press, Ithaca, N.Y.

Ballard, W. B., and R. W. Tobey. 1981. Decreased calf production of moose immobilized with anectine administered from helicopter. Wildl. Soc. Bull. 9:207–209.

Bamberg, E., and F. Schwarzenberg 1990. Fecal steroid assay for monitoring estrous cycle and pregnancy. IVth Congr. Int. Soc. Anim. Clin. Biochem., Davis, CA; 95–99.

Desaulniers, D. M., A. K. Goff, K. J. Betteridge, J. Rowell, and P. F. Flood. 1989. Reproductive hormone concentrations in faeces during the estrous cycle and pregnancy in cattle (Bos taurus) and muskoxen (Ovibus moschatus). Can. J. Zool. 67:1148–1154.

Kirkpatrick, J. F., C. B. Baker, J. W. Turner, Jr., R. M. Kenney, and V. K. Ganjam. 1979. Plasma corticosteroids as an index of stress in captive feral horses. J. Wildl. Manage. 43:801–804.

Kirkpatrick, J. F., L. H. Kasman, B. L. Lasley, and J. W. Turner, Jr. 1988. Pregnancy diagnosis in uncaptured feral horses. J. Wildl. Manage. 52:305–308.

Kirkpatrick, J. F., I. M. K. Liu, and J. W. Turner, Jr. 1990a. Remotely–delivered immunocontraception in feral horses. Wildl. Soc. Bull. 18:326–330.

Kirkpatrick, J. F., S. E. Shideler, and J. W. Turner, Jr. 1990b. Pregnancy determination in uncaptured feral horses based on free steroids in feces and steroid metabolites in urine–soaked snow. Can. J. Zool. 68:2576–2579.

Kirkpatrick, J. F., B. L. Lasley, and S. E. Shideler. 1990c. Urinary steroid evaluations to monitor ovarian function in exotic ungulates: VII. Urinary progesterone metabolites in the Equidae assessed by immunoassay. Zoo Biol. 9:341–348.

Kirkpatrick, J. F., V. Kincy, K. Bancroft, S. E. Shideler, and B. L. Lasley. 1991a. Oestrous cycle of the North American bison (*Bison bison*) characterized by urinary pregnanediol–3–glucuronide. J. Reprod. Fert. 93: (in press).

Kirkpatrick, J. F., S. E. Shideler, B. L. Lasley, and J. W. Turner, Jr. 1991b. pregnancy diagnosis in feral horses by means of fecal steroid conjugates. Theriogenology 35:753–759.

Kirkpatrick, J. F., and J. W. Turner, Jr. 1991c. Changes in herd stallions among feral horses bands and the absence of forced copulation and induced abortion. Behav. Ecol. Sociobiol. 29:217–220.

Kirkpatrick, J. F., and J. W. Turner, Jr. 1991d. Compensatory reproduction in feral horses. J. Wildl. Manage. 55:649–652.

Kirkpatrick, J. F., I. M. K. Liu, J. W. Turner, Jr., R. Naugle, and R. Keiper. 1992. Long–term effects of porcine zonae pellucidae immunocontraception on ovarian function in feral horses (Equus caballus). J. Reprod. Fert. 94:(in press).

Krasinski, Z., and J. Raczynski. 1967. The reproductive biology of European bison living in reserves and in freedom. Acta Theriologica 29:407–444.

Larsen, D. G., and D. A. Gauthier. 1989. Effects of capturing pregnant moose and calves on calf survivorship. J. Wildl. Manage. 53:564–567.

Lasley, B. L., and J. F. Kirkpatrick. 1991. Monitoring ovarian function in captive exotic species and free-roaming wildlife by means of urinary and fecal steroids. J. Zoo Wildl. Med. 22:23–31.

Loskutoff, N. M., J. E. Ott, and B. L. Lasley. 1983. Strategies for assessing ovarian function in exotic species. J. Zoo Anim. Med. 14:3–12.

Lasley, B. L. 1985. Methods for evaluating reproductive function in exotic species. Adv. Vet. Sci. Comp. Med. 30:209–228.

Mostl, E., H. S. Choi, W. Wurm, N. Ismail, and E. Bamberg. 1984. Pregnancy determination in cows and heifers by determination of oestradiol-17α in faeces. Brit. Vet. J. 140:287–291.

Munro, C. J., and G. H. Stabenfeldt. 1984. Development of a microtiter plate enzyme immunoassay for the determination of progesterone. J. Endocr. 101:41–49.

Safar-Hermann, N., M. N. Ismail, H. S. Choi, E. Mostl, and E. Bamberg. 1987. Pregnancy diagnosis in zoo animals by estrogen determination in feces. Zoo Biol. 6:189–193.

Schwarzenberger, F., E. Mostl, and E. Bamberg. 1990. Monitoring of the estrous cycle in farm animals by measuring gestagens in faeces. J. Steroid Biochem. 36:129.

Seal, U. S., S. M. Schmitt, and R. O. Peterson. 1985. Carfentanil and xylazine for immobilization of moose (Alces alces) on Isle Royale. J. Wildl. Dis. 21:48–51.

Shideler, S. E., L. Tell, G. Owiti, L. Laughlin, R. Chatterton, and B. L. Lasley. 1990. The relationship of serum estradiol and progesterone concentrations to the enzyme immunoassay measurement of urinary estrone conjugates and immunoreactive pregnanediol-3-glucuronide in *Macaca mulatta*. Am. J. Primatol. 22:113–122.

Valkenburg, P., R. O. Boertje, and J. L. Davis. 1983. Effects of darting and netting of caribou in Alaska. J. Wildl. Manage. 47:1233–1237.

Coping with Different Metabolic Strategies

Chris R. Pryce[*] and Max Döbeli[§]
[*]Anthropology Institute and Museum and
[§]Klinik für Andrologie und Gynäkologie,
Vet.-Med. Fakultät, University of Zürich, Zürich, Switzerland

Introduction

There are some 20 species of clawed monkey in South America, and with one exception these are the marmosets and tamarins that constitute the family Callitrichidae. The exception is the Goeldi's monkey (*Callimico goeldii*), a species belonging to a monospecific genus and regarded by some sources as the sole member of the family, Callimiconidae (Hershkovitz, 1977; Heltne et al., 1981). The status of a number of these species, including the Goeldi's monkey, is officially recognised as endangered (CITES, Appendix I), while some marmosets and tamarins represent valuable research models in biomedicine (Hearn, 1983). There are clear and justifiable grounds for wanting to understand the reproductive biology of the clawed New World monkeys, therefore. In contrast to many Old World primates, females of these species demonstrate no physical evidence of their reproductive state during the ovarian cycle, and behavioural correlates are, at best, subtle. Questions relating to reproductive state, including the presence of fertility, the timing of ovulation, and the onset of pregnancy, can only be answered via endocrine monitoring, therefore. This situation is reflected in the published studies of the reproductive endocrinology of the common marmoset (*Callithrix jacchus*) (Eastman et al., 1984; Harlow et al., 1983), the cotton-topped tamarin (*Saguinus oedipus*) (Ziegler et al., 1987), the golden-lion tamarin (*Leontopithecus rosalia*) (French & Inglett, 1985), and the Goeldi's monkey (Carroll et al., 1990), for example. With the exception of the common marmoset, all the endocrine information available is based on non-invasive studies, and these studies refer, with one exception, to the analysis of reproductive steroids (and peptides) measured in samples of urine. One single study to-date, in the cotton-topped tamarin, has investigated excretion of reproductive steroids in faeces (Ziegler et al., 1989).

Against this background, therefore, our objectives in the present study were to compare the concentrations of oestrone and oestradiol, and their metabolites, in the peripheral circulation, the urine and the faeces of fertile females from two species of clawed New World primate, the common marmoset and the Goeldi's monkey. We began with the hypotheses that, (i) comparison of the relative concentrations of different oestrogens pre- and post-excretion should provide us with indirect comparative information about oestrogen metabolism in these two species, and (ii) comparison between plasma, urinary and faecal oestrogens should provide us with direct information on the relative merits of different approaches to the non-invasive monitoring of reproductive state in our study species.

METHODS

Animals

The monkeys used were housed in the New World Primate Laboratory of the Anthropology Institute, University of Zürich. Sexually mature female common marmosets (n = 5) and Goeldi's monkeys (n = 5) were selected for study. Subjects were housed with a single sexually immature conspecific (n = 3, 3) or a mature male (n = 2, 2). The common marmosets lived indoors in mesh-wire cages measuring 1 x 2 x 2 m, and the Goeldi's monkeys in indoor cages measuring 2.2 x 1.1 x 2.3 m. All cages contained