DIAGNOSING PREGNANCY IN FREE-RANGING ELK USING FECAL STEROID METABOLITES

Authors: White, P. J., Garrott, Robert A., Kirkpatrick, Jay F., and Berkeley, Elizabeth V.

Source: Journal of Wildlife Diseases, 31(4) : 514-522

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-31.4.514

Journal of Wildlife Diseases, 31(4), 1995, pp. 514–522

DIAGNOSING PREGNANCY IN FREE-RANGING ELK USING FECAL STEROID METABOLITES

P. J. White,[1] Robert A. Garrott,[1] Jay F. Kirkpatrick,[2] and Elizabeth V. Berkeley[2]

[1] Department of Wildlife Ecology, University of Wisconsin–Madison, Madison, Wisconsin 53706, USA

[2] Deaconess Research Institute, 1500 Poly, Billings, Montana 59102, USA

ABSTRACT: We validated fecal metabolite analysis as a noninvasive means of diagnosing pregnancy in uncaptured, free-ranging Rocky Mountain elk (*Cervus elaphus nelsoni*). During November 1991, we collected blood samples from 21 radiocollared, 1- to 10-year-old female elk in Yellowstone National Park, Wyoming (USA), and determined their pregnancy status by radioimmunoassay of serum pregnancy specific protein B and serum progesterone concentrations. From December 1991 through April 1992, we collected three to 12 fecal samples from each collared elk and measured the concentration of estrone conjugates, pregnanediol-3-glucuronide, and free progesterone by enzyme immunoassays. We also evaluated fecal samples from 10 unmarked male and eight calf elk. Pregnant females had significantly ($P < 0.001$) higher concentrations of all three fecal metabolites than nonpregnant animals, especially later in gestation (March to April). We developed all possible combinations of univariate, bivariate, and multivariate discriminant function analysis models to determine those variables most useful in predicting memberships of pregnant versus nonpregnant elk during the March to April time-period. We validated each model by applying the classification functions to 11 pregnant and eight nonpregnant elk that were not included in the development of the original models. Accuracy of the discriminant function analysis models ranged from 57 to 84%, with the univariate model based on pregnanediol-3-glucuronide concentration providing the highest classification. Fecal metabolite analysis will enable biologists to noninvasively assess the pregnancy status of elk, especially when diagnoses are based upon multiple samples collected between mid-March and mid-April.

Key words: *Cervus elaphus*, elk, feces, enzyme immunoassays, pregnancy, reproduction, steroids.

INTRODUCTION

During pregnancy, the metabolites of reproductive steroids are excreted in feces (Desaulniers et al., 1989), and their concentrations can be measured to assess the pregnancy status of animals (Lasley, 1985). Recently, the concentrations of fecal metabolites such as estrone conjugates (E_1C), pregnanediol-3-glucuronide (PdG), or free progesterone (P_4) have been used to accurately diagnose pregnancy in several species of ungulates, including caribou (*Rangifer tarandus*) (Messier et al., 1990), moose (*Alces alces*) (Monfort et al., 1993) and feral horses (*Equus caballus*) (Lucas et al., 1991). Workers in these studies employed radioimmunoassay (RIA) procedures for quantifying fecal metabolites. However, enzyme immunoassay (EIA) procedures that provide the potential for less expensive and rapid, on-site diagnoses also are available (Munro and Stabenfeldt, 1984). Enzyme immunoassays have been used to diagnose pregnancy in free-ranging bison (*Bison bison*) (Kirkpatrick et al., 1993a) and feral horses (Kirkpatrick et al., 1990b), but cannot be used to diagnose pregnancy in elk until they have been validated and classification criteria are developed. Assay validation should include serial sampling of feces and comparison of the fecal data to independent and traditional methods of diagnosing pregnancy (RIA of serum pregnancy specific protein B [PSPB] or progesterone, rectal palpation, transrectal ultrasonography) (Lasley and Kirkpatrick, 1991). Our purpose was to evaluate the accuracy of using fecal metabolites to assess pregnancy status in elk using EIA procedures. Specific objectives were to determine if concentrations of fecal metabolites differed significantly between pregnant and nonpregnant animals during gestation, to determine the period during gestation when feces would most

accurately distinguish between pregnant and nonpregnant animals, and to produce classification functions that accurately predict the pregnancy status of elk.

MATERIALS AND METHODS

During November 1991, we radiocollared 21 one to 10-yr-old female elk in the Madison-Firehole drainage of Yellowstone National Park, Wyoming (USA) (44°37′N, 110°52′W). Craighead et al. (1973) provided a detailed description of the climate, fauna, and flora of the area. During handling, 20 ml of blood was drawn from each elk. Blood samples were centrifuged within 6 hr of sampling, and serum was drawn off and stored at −20 C. The pregnancy status of all collared elk was diagnosing using RIA of serum PSPB (Sasser et al., 1986), and then confirmed by RIA of serum progesterone (Plotka et al., 1975). The PSPB assay was developed for domestic cattle sera and, consequently, PSPB in elk serum could not be quantified. However, PSPB does provide a qualitative determination of pregnancy status since radioactivity (^{125}I-PSPB) bound to cattle PSPB antisera remains at or near 100% in sera from nonpregnant animals, but is inhibited (<93%) in sera from pregnant animals (Wood et al., 1986). Both the PSPB and P_4 assays have been validated in elk (Hein et al., 1991). Based on these studies, elk were considered to be pregnant if the serum sample bound <93% of the iodinated tracer in the PSPB assay and if serum progesterone concentrations were >1 ng/ml.

From December 1991 through April 1992, we located all collared elk at least once weekly and observed them at distances of 10 to 50 m for 30 min. If individually marked animals defecated, we collected several fecal pellets and stored them at −20 C in plastic bags until analysis. We collected 117 fecal samples from pregnant elk and 14 samples from nonpregnant elk (three to 12 fecal samples per animal). We also collected 10 fecal samples from unmarked male elk and eight samples from unmarked calf elk that we opportunistically observed defecating. During March 1993, we obtained additional fecal samples from ten 1- to 8-year-old free-ranging female elk in Fitzner/Eberhardt Arid Lands Ecology Reserve, Washington (USA) (46°25′N, 119°33′W) and nine 1- to 13-yr-old captive female elk at the Bronx Zoo, New York, New York (USA). The pregnancy status of free-ranging elk in Washington was determined using rectal palpation and RIA of serum PSPB and P_4. Eight of the captive elk had been treated with the contraceptive porcine zona pellucida (PZP) vaccine (Kirkpatrick et al., 1990a). This vaccine works by blocking fertilization (Sacco et al., 1984) and does not interfere with pregnancies underway (Kirkpatrick et al., 1990a). We used the delivery of a calf to confirm pregnancy in captive elk treated with the PZP vaccine, and in free-ranging elk in Yellowstone National Park.

Steroid metabolites were extracted from fecal samples with an aqueous extraction buffer (Shideler et al., 1993). A single fecal pellet from each sample was thawed, weighed, and suspended in 5.0 ml of 0.1 M, pH 7.0 extraction buffer with 0.1% BSA (Fraction V, CalBiochem, La Jolla, California, USA) in a pre-weighed scintillation vial. Fecal pellets were broken apart and shaken (120 rpm) for 18 hr at 18 C. The liquid then was decanted and centrifuged (1200 × *G*) for 10 min. The wet fecal matter from each pellet was air-dried at 20 C until weight was constant to determine the equivalent dry weight.

Concentrations of fecal steroids were measured using the EIA techniques described by Munro and Stabenfeldt (1984) for P_4, Kirkpatrick et al. (1991) for PdG, and Munro et al. (1991) for E_1C. Briefly, we prepared flat-bottomed, polystyrene, microtiter plates (96-well) by coating them with 50 µl per well of antibody. We used antibody R522 in coating buffer at a dilution of 1:10 for E_1C EIA, antibody P-70 at a dilution of 1:50 for PdG EIA, and antibody 4861 at a dilution of 1:10 for P_4 EIA. Antibodies were prepared as described in Munro and Lasley (1988) by Dr. B. L. Lasley, Institute of Toxicology and Health Research, University of California, Davis, California. Plates were sealed tightly with an acetate sealer and incubated for a minimum of 12 hr at 4 C. After incubation, the wells of each microtiter plate were emptied by inversion, washed four times with a solution of 1.5 M NaCl and 5% Tween-20 in distilled water, and drained to remove antiserum not bound to polystyrene. Fifty µl EIA buffer was added to all wells for insulation purposes, and plates were sealed and equilibrated for 2 hr at 18 C. We then added 50 µl of horseradish peroxidase steroid conjugate at a dilution of 1:20,000 in EIA buffer to all wells, sealed the plates, and incubated them for 2 hr at 18 C. After incubation, the plates were washed as described above and 20 µl of extract supernatant was added to each well. Plates were sealed, and shaken for 30 to 60 min until color developed. Absorbance was measured by the Dynatech MR5000 microplate reader (Dynatech Lab., Inc., Chantilly, Virginia, USA) with a test filter of 405 nm and a reference filter of 407 nm. Metabolite concentrations are expressed as ng/g dry feces.

To generate standard curves, we added 20 µl of distilled water to four wells per plate, and 50 µl of standards to 20 wells per plate. Standard concentrations ranged from 6.25 to 200 pg per

well for E_1C, 0.078 to 10 ng per well for PdG, and 15.6 to 2,000 pg per well for P_4. A standard dose-response curve was constructed by plotting the percent bound against the amount of added E_1C, PdG, or P_4. Parallelism to the standard curve was determined by successively halving the dilutions of four fecal samples and graphing the assay results against the standard curve. Serial dilutions had parallelism with the standard curve for each of the three assays. The inter-assay coefficients of variation were 8% ($n = 4$) for E_1C, 15% ($n = 7$) for PdG, and 16% ($n = 7$) for P_4. Intra-assay coefficients of variation were 10% ($n = 7$) for E_1C, 8% ($n = 10$) for PdG, and 9% ($n = 7$) for P_4. Assay sensitivity was 6.3 pg per well for E_1C, 70.0 pg per well for PdG, and 15.6 pg per well for P_4. Recovery of E_1C, PdG, and P_4 was previously determined by adding known quantities of ^{3}H-estrone sulfate, ^{3}H-PdG, and ^{3}H-P_4 to the wet samples, extracting the samples as described above, and comparing label recovered to label added. Recovery was 72% for E_1C, 62% for PdG, and 51% for P_4. Results were not corrected for recoveries.

We divided the winter into three sampling periods so that at least one fecal sample per elk occurred during each period. Thus, samples were classified as having been collected during early (December to January; elk 3- to 4-mo pregnant), middle (February; elk 5-mo pregnant), or late (March to April, elk 6- to 7-mo pregnant) winter. Next, we used a $\log_e$-transformation on the steroid metabolite data from elk in Yellowstone National Park (original data set) and randomly selected one sample per elk per sampling period to develop a data set comprised of independent samples drawn from a population with a multivariate normal distribution. Multivariate analysis of variance (MANOVA) (Sokal and Rohlf, 1981) was used to test the null hypothesis that no differences in fecal metabolite concentrations existed among males, calves, nonpregnant females, and pregnant females.

Data from groups with comparable fecal metabolite concentrations were combined, and discriminant function analysis (DFA) (Klecka, 1980) based upon the three fecal metabolite concentrations was conducted to determine the sampling period when feces should be collected to most accurately distinguish between samples from pregnant and nonpregnant animals. We chose the most favorable sampling period based on multivariate measures of group differences (Wilks' λ, canonical correlation) (Klecka, 1980) over the discriminating variables. Next, we computed the mean metabolite concentrations for each elk during the most favorable sampling period. We conducted DFA on the mean, transformed data to define and predict group memberships of pregnant and nonpregnant elk. We developed all possible combinations of univariate, bivariate, and multivariate models to determine those variables most useful in predicting memberships of pregnant versus nonpregnant elk. We validated each model by applying the classification functions to 11 pregnant and eight nonpregnant elk that were not included in the development of the original models. Errors in classification were used to demonstrate how well each model performed.

RESULTS

Radioactivity bound to cattle PSPB antisera had a mean (± SE) of 73 (± 1)%, with a maximum of 84%, in pregnant females, and remained at 100% in all nonpregnant females. Mean ± SE serum P_4 levels were 3.2 ± 0.4 ng/ml (range = 1.4 to 7.5 ng/ml) in pregnant females and 0.1 ± 0.0 ng/ml (range, 0 to 0.13 ng/ml) in nonpregnant females. Eighteen of 21 collared elk in Yellowstone National Park were pregnant; serum PSPB and P_4 results were in full agreement and 17 of these elk were observed nursing, grooming, or defending calves during the following summer. Based on rectal palpation and serum PSPB and P_4 assays, all 10 elk from Washington were pregnant. None of the eight captive elk (Bronx Zoo) treated with the PZP vaccine produced calves, but the one untreated elk did produce a calf.

Differences among pregnant and nonpregnant groups existed for each of the three fecal metabolites ($P < 0.001$). Pregnant females had higher concentrations of fecal P_4 and PdG than nonpregnant elk during each sampling period ($P < 0.003$), and higher concentrations of fecal E_1C during the late sampling period ($P = 0.02$) (Table 1). However, pregnant and nonpregnant animals had similar concentrations of fecal E_1C during the early (December and January) and middle (February) sampling periods ($P > 0.38$) (Table 1). Thus, differences in fecal metabolite concentrations among pregnant and nonpregnant groups were greatest during the late sampling period ($P < 0.0001$) (Fig. 1).

Males had significantly ($P < 0.01$) lower concentrations of PdG than calves and

TABLE 1. Pooled steroid concentrations (ng/g dry fecal weight) of estrone conjugates (E_1C), pregnanediol-3-glucuronide (PdG), and free progesterone (P_4) in feces collected from elk in Yellowstone National Park, Wyoming ($n = 21$), the Arid Lands Ecology Reserve, Washington ($n = 10$), and the Bronx Zoo, New York ($n = 9$) during December 1991 through April 1992. Data include both original and validation sets. The results of a multivariate analysis of variance (F) also are provided.

	Pregnant females			Nonpregnant females			Males			Calves			
Variable	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	F
December to January													
E_1C	4.9	0.5	18	3.2	0.6	3	4.0	0.6	4	3.3	0.5	2	1.1
PdG	325.9[a]	30.3	18	192.8	21.9	3	63.4[b]	10.6	4	125.4	6.7	2	13.9
P_4	734.4[a]	66.3	18	82.5	18.0	3	317.5	138.6	4	300.9	8.4	2	7.6
February													
E_1C	5.9	1.5	18	3.1	0.3	3	2.6	0.3	3	2.7	0.0	2	0.7
PdG	373.7[a]	31.8	18	172.9	36.8	3	55.1[b]	3.1	3	106.0	22.7	2	29.4
P_4	862.6[a]	84.7	18	184.6	126.0	3	148.2	15.4	3	333.3	83.3	2	6.5
March to April													
E_1C	36.8[a]	8.3	29	19.0	6.6	11	3.2	0.3	3	5.7	1.0	4	3.8
PdG	479.0[a]	24.7	29	169.2	16.0	11	95.4	8.4	3	130.4	16.3	4	14.6
P_4	1,181.6[a]	70.7	29	303.4	68.1	11	241.8	10.5	3	449.7	82.0	4	17.7

[a] Steroid concentrations annotated with a superscript (a) were significantly ($P < 0.05$) higher in pregnant animals as compared to other classes.
[b] Steroid concentrations annotated with a superscript (b) were significantly ($P < 0.05$) lower in males as compared to other classes.

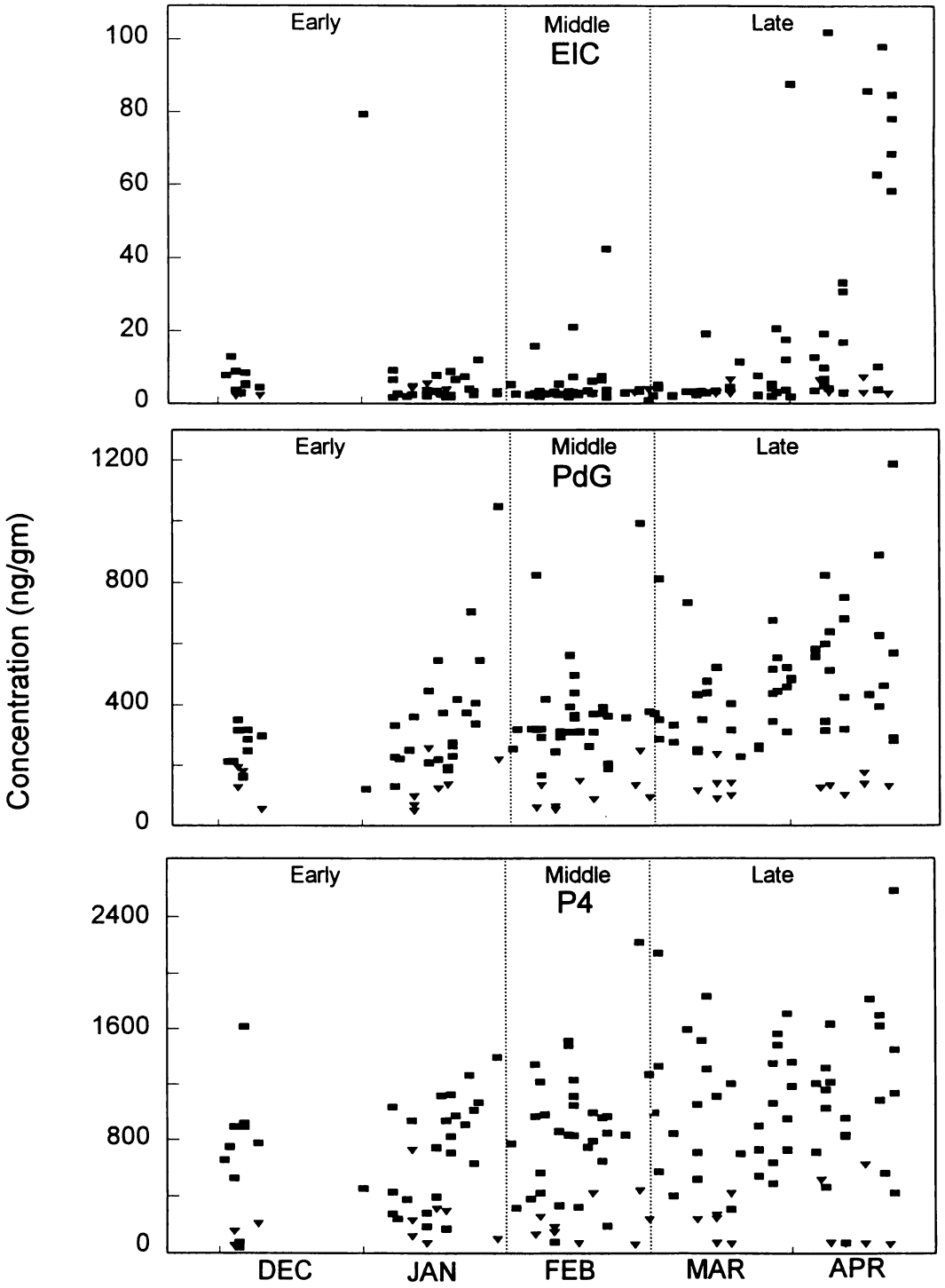

FIGURE 1. Temporal trend in the concentrations (ng/g dry fecal weight) of estrone conjugate (E_1C), pregnanediol-3-glucuronide (PdG), and progesterone (P_4) in dried feces (n = 149) of pregnant (squares) and nonpregnant (triangles) elk in Yellowstone National Park, Wyoming.

nonpregnant females during the early and middle sampling periods (Table 1). However, concentrations of fecal PdG were similar among these groups during the late sampling period ($P = 0.15$) (Table 1). Concentrations of fecal P_4 and E_1C were similar among males, calves, and nonpregnant females during each sampling period ($P > 0.05$) (Table 1), and we were unable to distinguish among these groups using DFA. Thus, we combined data from calves, males, and nonpregnant females into a nonpregnant group prior to developing DFA models.

Discriminant separation among pregnant and nonpregnant groups was least during the early sampling period (Wilks' $\lambda = 0.39$, canonical correlation = 0.78), greater during the middle sampling period ($\lambda = 0.32$, canonical correlation = 0.82), and greatest during the late sampling period ($\lambda = 0.19$, canonical correlation = 0.90). Thus, we defined and predicted group memberships of elk using fecal metabolite data collected during the late sampling period. Group membership largely could be predicted by PdG concentrations alone since all 11 of the pregnant elk and five of eight nonpregnant elk in our validation data set were correctly classified using this single variable (Table 2). Elk ranging in age from 1- to 13-yr-old were correctly classified using this variable. The three misclassified elk were 2-, 6- and 13-yr old, respectively. Concentrations of PdG above and below 223 ng/g equivalent dry weight were evidence of pregnancy and nonpregnancy (calf, male, or nonpregnant female), respectively. Bivariate and multivariate models provided overall classification accuracies ≥73% (Table 2), but did not improve classification accuracy as compared to the univariate PdG model.

DISCUSSION

To determine if fecal steroid metabolites accurately diagnosed pregnancy in elk, we first needed to conclusively determine whether each sampled elk was pregnant. We accomplished this by diagnosing and then confirming pregnancy using independent methods such as rectal palpation, RIA of PSPB or P_4, and the delivery of a calf. Rectal palpation of fetal membranes, uterine cotyledons, or fetal ballottement has been used to diagnose pregnancy in elk with reliable results (Hein et al., 1991). The PSPB assay is a highly accurate method of pregnancy detection in ungulates, including deer (*Dama dama, Odocoileus hemionus, O. virginianus*) (Wood et al., 1986; Wilker et al., 1993), elk (Haigh et al., 1988), and mountain goats (*Oreamnos americanus*) (Houston et al., 1986). The P_4 assay also is effective for diagnosing pregnancy in ungulates, including bighorn sheep (*Ovis canadensis*) (Ramsay and Sadleir, 1979), deer (Plotka et al., 1977a, b) and elk (Willard et al., 1994), although at times its reliability may not be as high as PSPB since concentrations in nonpregnant animals often are similar to those found in pregnant animals during the luteal phase of the estrous cycle (Plotka et al., 1977a, b) and the adrenal gland has been implicated as a significant source of P_4 in deer (Wesson et al., 1979; Plotka et al., 1983). Thus, a P_4 level from a single blood sample may not always be reliably used to assess pregnancy status (Plotka et al., 1983). For this reason, we only used P_4 to confirm pregnancy after an initial diagnosis by PSPB. Since the results of our independent tests were in full agreement, we were confident that the pregnancy status of each elk used in the study was accurately assessed.

Based on the enzyme immunoassays of fecal PdG, this metabolite has potential for being used to noninvasively assess the pregnancy status of elk. However, PdG concentrations still had some overlap between pregnant and nonpregnant elk during March and April. Thus, EIA of PdG may not always be a reliable sole indicator of pregnancy status in this species. Radioimmunoassay may provide better discrimination among groups since Messier et al. (1990) obtained complete separation among pregnant and nonpregnant caribou

TABLE 2. Accuracy of predicted group memberships of pregnant and nonpregnant elk with univariate, bivariate, and multivariate discriminant function analysis models based on steroid concentrations of estrone conjugates (E_1C), pregnanediol-3-glucuronide (PdG), and free progesterone (P_4) in 60 feces collected from 21 female elk in Yellowstone National Park, Wyoming (original data set) and 19 feces collected from 19 female elk in the Arid Lands Ecology Reserve, Washington or the Bronx zoo, New York (validation data set) during March and April 1992. The results for the original and validation data sets are presented as the overall percentage of known pregnant and nonpregnant elk correctly classified to their respective groups, as well as the proportion of each group that was correctly classified.

	Validation data set[a]			Original data set		
Models	Overall correct (%)	Sensitivity[b] (%)	Specificity[c] (%)	Overall correct (%)	Sensitivity (%)	Specificity (%)
Multivariate						
E_1C, PdG, P_4	84 (16/19)[d]	100 (11/11)	63 (5/8)	100 (60/60)	100 (54/54)	100 (6/6)
Bivariate						
E_1C, PdG	84 (16/19)	100 (11/11)	63 (5/8)	100 (60/60)	100 (54/54)	100 (6/6)
E_1C, P_4	74 (14/19)	100 (11/11)	38 (3/8)	93 (56/60)	93 (50/54)	100 (6/6)
PdG, P_4	84 (16/19)	100 (11/11)	63 (5/8)	100 (60/60)	100 (54/54)	100 (6/6)
Univariate						
E_1C	58 (11/19)	100 (11/11)	0 (0/8)	48 (29/60)	43 (23/54)	100 (6/6)
PdG	84 (16/19)	100 (11/11)	63 (5/8)	98 (59/60)	100 (54/54)	83 (5/6)
P_4	74 (14/19)	100 (11/11)	38 (3/8)	93 (56/60)	93 (50/54)	100 (6/6)

[a] The original data set was used to derive classification functions by discriminant function analysis. The validation data set was then used to test how well these functions performed (Klecka, 1980).
[b] Sensitivity is the proportion of true positives (pregnant elk) that are correctly identified by the test (Altman, 1991).
[c] Specificity is the proportion of true negatives (nonpregnant elk) that are correctly identified by the test (Altman, 1991).
[d] Percent correct (number correctly classified/actual number in group).

(*Rangifer tarandus*) using this technique. However, RIAs are more expensive than EIAs, require more sophisticated equipment compared with EIAs, and necessitate the disposal of radioactive material (Munro and Stabenfeldt, 1984). Also, rapid, on-site pregnancy diagnoses are possible with EIA (Kirkpatrick et al., 1993b), and newly developed extraction procedures may improve the discrimination of this technique.

Previous workers have diagnosed pregnancy status in ungulates by measuring the concentrations of several fecal metabolites during pregnancy and then using the metabolite that seemed most reliable as the sole indicator to classify animals. This technique works well if a single metabolite provides complete discrimination among pregnant and nonpregnant groups (Messier et al., 1990). However, if single metabolites do not provide complete discrimination among groups, then diagnostic techniques that use combinations of metabolites to predict pregnancy status may provide better discrimination. For example, Monfort et al. (1993) were 85 and 58% effective at predicting pregnancy status in moose (*Alces alces*) using fecal progesterone and estradiol concentrations, respectively. Perhaps their classification accuracy could have been improved by using a model based on the concentrations of both metabolites. Although bivariate and multivariate models did not improve classification accuracy during this study, we still recommend using DFA to determine the best combination of metabolites (univariate, bivariate, multivariate models) for pregnancy diagnosis in other species. This technique provides a less subjective, more thorough, and more accurate classification of pregnant and nonpregnant animals than classification techniques based solely on visual inspection of the data.

Fecal metabolite analysis will enable biologists to accurately and noninvasively assess the pregnancy status of individual elk, as well as the incidence of pregnancy in

elk populations. When coupled with observational data, fecal samples also could be used to assess demographic parameters that previously were extremely difficult to measure, such as calf production, embryonic and fetal mortality, and calf mortality (Messier et al., 1990). Estimations of juvenile mortality may be particularly valuable since juvenile survival is believed to be the most sensitive demographic parameter to change in response to changes in resource availability for many mammalian species (Eberhardt and Siniff, 1977). Thus, fecal metabolite analysis may be a valuable tool for monitoring the population dynamics of elk. However, researchers must ensure that fecal samples are collected only from mature female elk since samples from males and calves are not distinguishable from those of nonpregnant elk and will confound analyses and interpretations. Furthermore, we recommend that biologists collect several samples between mid-March and mid-April to assess pregnancy status of marked individuals, or the incidence of pregnancy in populations, since metabolite concentrations may vary somewhat among samples from the same individual.

ACKNOWLEDGMENTS

The Deaconess Research Institute, National Park Service, and University of Wisconsin financed our study. We thank L. Cadwell and B. Tiller of Pacific Northwest Laboratory for data on elk at the Fitzner/Eberhardt Arid Lands Ecology Reserve, J. Cary, D. Heisey, M. Newton, G. Sargeant, and C. A. V. White for statistical advice or comments on earlier drafts of this manuscript; E. Plotka and R. Sasser for serum assays; and R. Abegglen, P. Gogan and J. Varley for their encouragement and logistic support.

LITERATURE CITED

ALTMAN, D. G. 1991. Practical statistics for medical research. Chapman and Hall, New York, New York, 611 pp.

CRAIGHEAD, J. J., F. C. CRAIGHEAD, JR., R. L. RUFF, AND B. W. O'GARA. 1973. Home ranges and activity patterns of nonmigratory elk of the Madison drainage herd as determined by biotelemetry. Wildlife Monograph 33, The Wildlife Society, Washington, D.C., 50 pp.

DESAULNIERS, D. M., A. K. GOFF, K. J. BETTERIDGE, J. ROWELL, AND P. F. FLOOD. 1989. Reproductive hormone concentrations in faeces during the oestrous cycle and pregnancy in cattle (*Bos taurus*) and muskoxen (*Ovibos moschatus*). Canadian Journal of Zoology 67: 1148–1154.

EBERHARDT, L. L., AND D. B. SINIFF. 1977. Population dynamics and marine mammal management policies. Journal of the Fisheries Research Board of Canada 34: 183–190.

HAIGH, J. C., M. CRANFIELD, AND R. G. SASSER. 1988. Estrus synchronization and pregnancy diagnosis in red deer. Journal of Zoo Animal Medicine 19: 202–207.

HEIN, R. G., J. L. MUSSER, AND E. F. BRACKEN. 1991. Serologic, parasitic and pregnancy survey of the Colockum elk herd in Washington. Northwest Science 65: 217–222.

HOUSTON, D. B., C. T. ROBBINS, C. A. RUDER, AND R. G. SASSER. 1986. Pregnancy detection in mountain goats by assay for pregnancy-specific protein B. The Journal of Wildlife Management 50: 740–742.

KIRKPATRICK, J. F., I. K. N. LIU, AND J. W. TURNER, JR. 1990a. Remotely-delivered immunocontraception in feral horses. Wildlife Society Bulletin 18: 326–330.

———, 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. Canadian Journal of Zoology 68: 2576–2579.

———, V. KINCY, K. BANCROFT, S. E. SHIDELER, AND B. L. LASLEY. 1991. The estrous cycle of the North American bison (*Bison bison*) characterized by urinary pregnanediol-3-glucuronide. Journal of Reproduction and Fertility 92: 541–547.

———, D. F. GUDERMUTH, R. L. FLAGEN, J. C. MCCARTHY, AND B. L. LASLEY. 1993a. Remote monitoring of ovulation and pregnancy of Yellowstone bison. The Journal of Wildlife Management 57: 407–412.

———, B. L. LASLEY, S. E. SHIDELER, J. F. ROSER, AND J. W. TURNER, JR. 1993b. Non-instrumented immunoassay field tests for pregnancy detection in free-roaming feral horses. The Journal of Wildlife Management 57: 168–173.

KLECKA, W. R. 1980. Discriminant analysis. Sage Publications, Inc., Newbury Park, California, 71 pp.

LASLEY, B. L. 1985. Methods for evaluating reproductive function in exotic species. Advances in Veterinary Science and Comparative Medicine 30: 209–228.

———, AND J. F. KIRKPATRICK. 1991. Monitoring ovarian function in captive and free-ranging wildlife by means of urinary and fecal steroids. Journal of Zoo and Wildlife Medicine 22: 22–31.

LUCAS, Z., J. I. RAESIDE, AND K. J. BETTERIDGE. 1991.

Non-invasive assessment of the incidences of pregnancy and pregnancy loss in the feral horses of Sable Island. Journal of Reproduction and Fertility (Supplement 44): 479–488.

MESSIER, F., D. M. DESAULNIERS, A. K. GOFF, R. NAULT, R. PATENAUDE, AND M. CRETE. 1990. Caribou pregnancy diagnosis from immunoreactive progestins and estrogens excreted in feces. The Journal of Wildlife Management 54: 279–283.

MONFORT, S. L., C. C. SCHWARTZ, AND S. K. WASSER. 1993. Monitoring reproduction in captive moose using urinary and fecal steroid metabolites. The Journal of Wildlife Management 57: 400–407.

MUNRO, C., AND B. L. LASLEY. 1988. Non-radiometric methods for immunoassay of steroid hormones. *In* Polypeptide and steroid hormone detection. F. Hazeltine and B. Albertson (eds.). Alan Liss, Inc., New York, New York, pp. 289–329.

———, AND G. STABENFELDT. 1984. Development of a microtitre plate enzyme immunoassay for the determination of progesterone. Journal of Endocrinology 101: 41–49.

———, ———, J. R. CRAGUN, L. A. ADDIEGIO, L. S. SHAHNAZARIAN, J. W. OVERSTREET, AND B. L. LASLEY. 1991. Relationship of serum estrogen and progesterone concentrations to the excretion profiles of their major metabolites as measured by enzyme immunoassay. Clinical Chemistry 37: 838–844.

PLOTKA, E. D., C. W. FOLEY, D. M. WITHERSPOON, G. C. SCHMOLLER, AND D. D. GOETSCH. 1975. Periovulatory changes in peripheral plasma progesterone and estrogen concentrations in the mare. American Journal of Veterinary Research 36: 1359–1362.

———, U. S. SEAL, L. J. VERME, AND J. J. OZOGA. 1977a. Reproductive steroids in the white-tailed deer (*Odocoileus virginianus borealis*). II. Progesterone and estrogen levels in peripheral plasma during pregnancy. Biology of Reproduction 17: 78–83.

———, U. S. SEAL, G. C. SCHMOLLER, P. D. KARNS, AND K. D. KEENLYNE. 1977b. Reproductive steroids in the white-tailed deer (*Odocoileus virginianus borealis*). I. Seasonal changes in the female. Biology of Reproduction 16: 340–343.

———, ———, ———, AND ———. 1983. The adrenal gland in white-tailed deer: A significant source of progesterone. The Journal of Wildlife Management 47: 38–44.

RAMSAY, M. A., AND R. M. F. S. SADLEIR. 1979. Detection of pregnancy in living bighorn sheep by progestin determination. The Journal of Wildlife Management 43: 970–973.

SACCO, A. G., M. G. SUBRAMANIAN, AND E. C. YUREWICZ. 1984. Association of sperm receptor activity with a purified pig zona antigen (PPZA). Journal of Immunology 6: 89–103.

SASSER, R. G., C. A. RUDER, K. A. IVANI, J. E. BUTLER, AND W. C. HAMILTON. 1986. Detection of pregnancy by radioimmunoassay of a novel pregnancy-specific protein in serum of cows and a profile of serum concentrations during gestation. Biology of Reproduction 35: 936–942.

SHIDELER, S. E., A. M. ORTUNO, F. M. MORAN, E. A. MOORMAN, AND B. L. LASLEY. 1993. Simple extraction and enzyme immunoassays for estrogen and progesterone metabolites in the feces of *Macaca fascicularis* during non-conceptive and conceptive ovarian cycles. Biology of Reproduction 48: 1290–1298.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry. W. H. Freeman and Company, New York, New York, 859 pp.

WESSON, J. A., III, P. F. SCANLON, R. L. KIRKPATRICK, H. S. MOSBY, AND R. L. BUTCHER. 1979. Influence of chemical immobilization and physical restraint on steroid hormone levels in blood of white-tailed deer. Canadian Journal of Zoology 57: 768–776.

WILKER, C., B. BALL, T. REIMERS, G. SASSER, M. BRUNNER, B. ALEXANDER, AND M. GIAQUINTO. 1993. Use of pregnancy-specific protein-B and estrone sulfate for determination of pregnancy on day 49 in fallow deer (*Dama dama*). Theriogenology 40: 307–312.

WILLARD, S. T., R. G. SASSER, J. C. GILLESPIE, J. T. JAQUES, T. H. WELSH, JR., AND R. D. RANDEL. 1994. Methods for pregnancy determination and the effects of body condition on pregnancy status in Rocky Mountain elk (*Cervus elaphus nelsoni*). Theriogenology 42: 1095–1102.

WOOD, A. K., R. E. SHORT, A. DARLING, G. L. DUSEK, R. G. SASSER, AND C. A. RUDER. 1986. Serum assays for detecting pregnancy in mule and white-tailed deer. The Journal of Wildlife Management 50: 684–687.

Received for publication 29 April 1994.