

Use of Fecal Steroid Metabolites to Estimate the Pregnancy Rate of a Free-Ranging Herd of Tule Elk

Author(s): Monica A. Stoops, Gary B. Anderson, Bill L. Lasley, Susan E. Shideler

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USE OF FECAL STEROID METABOLITES TO ESTIMATE THE PREGNANCY RATE OF A FREE-RANGING HERD OF TULE ELK

MONICA A. STOOPS, Department of Animal Science, University of California, Davis, CA 95616, USA

GARY B. ANDERSON, Department of Animal Science, University of California, Davis, CA 95616, USA

BILL L. LASLEY, Institute of Toxicology and Environmental Health, University of California, Davis, CA 95616, USA

SUSAN E. SHIDLER,¹ Institute of Toxicology and Environmental Health, University of California, Davis, CA 95616, USA

Abstract: The ability to measure reproductive hormones in urine and feces permits physiologic evaluations of free-ranging animal populations. The data from previous reports indicate specific events such as gonadal recrudescence, ovulation, conception, pregnancy, and lactation can be detected and defined by fecal hormone analysis in a wide range of animals. The present study was undertaken to determine if combined data from a longitudinal and cross-sectional hormonal study of free-ranging cow tule elk (*Cervus elaphus nannodes*) and simultaneously collected observational data would permit an accurate estimation of the pregnancy rate of the population. Ovarian and placental function were monitored in 34 radiocollared cow tule elk from the onset of ovarian recrudescence in the summer of 1996, through the calving season in the spring of 1997. Estrogen and progesterone metabolites were measured by enzyme immunoassay (EIA) in fecal samples collected from both radiocollared and uncollared cows. The radiocollared cows were located and identified for individualized observations of fecal deposition and later confirmation of pregnancy by observation of nursing. Hormonal concentrations from the samples collected from the radiocollared cows in which pregnancy and outcome were known were used to set criteria for designation of a cow as pregnant in the cross-sectional samples collected from a population of approximately 208 uncollared cows. Progesterone metabolite (PdG) concentrations were 100% reliable at predicting pregnancy within the first trimester of gestation through parturition, while estrogen metabolite (E₁C) concentrations were not a reliable indicator until the last trimester of gestation. A progesterone metabolite concentration ≥ 1.01 ug/g dry mass feces (dmf) after the breeding season was set as confirmation of pregnancy status in fecal samples collected from uncollared cows. Analysis of hormonal concentrations after the 1997 calving season in conjunction with observations of nursing permitted pregnancy detection estimates via the calving rate of the collared cows for the 1996–97 breeding and calving season. These same parameters were estimated and evaluated in the uncollared cows. Our results provide strong evidence that the measure of fecundity in free-ranging animals can be obtained through fecal steroid analyses, and that this measure is an accurate predictor of fertility.

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The fact that the structure of gonadal and placental steroid hormones appears to be ubiquitous across mammals permits the development of methods to quantify reproductive hormones for accurate assessment of reproductive

status in a wide array of animal species. Much information has been obtained from studies conducted on captive wildlife groups; however, information is more limited on free-ranging species because of the difficulty of specimen collection. Serial samples are required for accurate hormonal identification of reproductive

¹ E-mail: seshideler@ucdavis.edu

events. Invasive methods of sampling for the assessment and monitoring of reproductive status do not lend themselves to repetitive sampling in wild animals. For example, serial blood sampling has proven potentially unsafe and unreliable in wild animals due to the need for repeated chemical immobilization or physical restraint of the animal (Kirkpatrick et al. 1979, Warren and Robert 1981, Plotka et al. 1983, Valkenburg et al. 1983, Asher et al. 1989, Larsen and Gauthier 1989). Noninvasive methods for characterizing the reproductive life history of nondomestic species and monitoring their reproductive status have been developed via hormonal metabolites found in urine and feces. Excreted metabolites of reproductive hormones permit physiologic evaluations without manipulating or stressing the animal, thus more accurately reflecting a natural hormonal state.

Urine and fecal sampling have been used by researchers to define and monitor reproductive events in an ever-expanding list of wild animals. The ability to collect noninvasive, repetitive biological samples containing hormonal information makes urine and fecal sampling superior to the invasive methods shown to compromise the physiological state of wild animals. This sampling approach has allowed free-ranging animals to be subjected to long-term hormonal analysis that was not practical with previous methods. Through such longitudinal studies, the reproductive biology of free-ranging species can be better understood because at least 5 characteristics of female reproduction can be evaluated through the measurement of fecal steroids: ovarian recrudescence, estrus, conception (fecundity), fetal loss, and parturition (fertility). Thus, difficult demographic parameters such as calf production, fetal mortality, and neonatal mortality can be more accurately assessed and incorporated into management plans.

The objective of this study was to determine the feasibility of using fecal steroid measurements as a method to monitor ovarian and placental activity in a free-ranging herd of tule elk. Specifically, we examined the hormonal profiles of radiocollared cows subjected to serial fecal collections to establish classification criteria (pregnant vs. nonpregnant). Additionally, we used this information to estimate the pregnancy rate of the herd from fecal samples collected from uncollared cows.

METHODS

Species, Sampling, and Demographic Data Collection

Animals ($n = 208$) in this study were from a population of tule elk ($n = 470$) located at Point Reyes National Seashore, California. The population of tule elk at Point Reyes National Seashore was established in 1978 as part of a program to relocate and reestablish tule elk herds in historic range throughout California. Several tule elk from the San Luis Island National Wildlife Refuge were reintroduced into a 1,040-ha area in Point Reyes National Seashore geographically known as Tomales Point. The founder population consisted of 10 adult elk (8 cows, 2 bulls).

Initial population growth was slow due to prior dairy cattle grazing on the area and direct loss of individual herd members. With improvements in range conditions in following years, the tule elk population at Tomales Point has grown, with the highest annual rate of increase (33%) occurring over the past 2 years (1996–97; J. A. Howell, U.S. Geological Survey, Biological Resources Division [USGS-BRD], personal communication). Due to heavy rainfall following 6 years of drought, tule elk at Point Reyes National Seashore exhibited extended breeding (Jun–Nov) and calving seasons (Feb–Aug) during 1996 and 1997. Cows generally give birth to a single calf approximately 250 days following a successful breeding (McCullough 1969, Thomas and Toweill 1982).

We captured adult cows (20 in 1995, 18 in 1996) via ballistic nets fired from a helicopter, followed by physical restraint of the animal. At the time of capture, cows were fitted with radiocollars containing an individualized tricolored banding pattern. During processing of animals, blood and fecal samples were collected, after which cows were released. We used 34 radiocollared cows in this study for longitudinal fecal hormone analysis, while we collected cross-sectional samples from a population of 208 uncollared cows.

From July 1996 through July 1997, all radiocollared cows were located at least once monthly and observed at distances of 14–364 m. If individually identified animals defecated, we noted the location of defecation and used a range finder to determine the distance that the animal was from the observer at the time and place of defecation. The person collecting fecal

samples was directed to the site by a second person using hand-held radios. In general, we located >1 but not >10 radiocollared cows in a particular group under observation. Therefore, time was recorded and location maps and distances to the individual fecal samples were made to maximize the number of fecal samples collected from radiocollared cows in a group. The mean number of fecal samples collected per radiocollared cow was 14.0 for cows captured in 1995 (range = 7.0–18.0) and 7.0 for cows captured in 1996 (range = 5.0–12.0). We also collected fecal samples from uncollared cows opportunistically observed defecating. Once again, location maps for fecal samples were made for a particular group under observation. Samples obtained from uncollared cows represented population samples and were grouped according to the month of collection. At least 5.0 g of fecal matter was collected and placed into an appropriately marked plastic bag. Each bag contained the identification of the cow from which the sample was collected, the date, and location of collection. Samples were stored initially on ice and then frozen (-20°C) until later analysis. All fecal samples were collected within 2 hr of defecation and frozen within 12 hr.

Confirmation of a calf was determined by visual observance of nursing. Attempts were made to collect fecal samples from each radiocollared cow following calving. In those radiocollared cows where the fecal hormonal profile confirmed pregnancy but no visual confirmation of a calf was noted, fecal samples were collected to confirm calving based on hormonal metabolite concentrations.

Sample Preparation

We thawed frozen fecal samples by placing them at refrigerator temperature (4°C) for 24 hr. After fecal samples were thawed, 5.0 g of fecal matter were put into a glass scintillation vial and placed into a drying (36°C) oven for approximately 62 hr. Every 24 hr, the fecal matter in each scintillation vial was crushed manually by use of an aluminum spatula to break up the fecal mass for subsequent solubilization. Final dryness was determined when the mass of samples remained constant and no longer measured a decrease in mass with continued drying.

Dried fecal samples were solubilized to extract steroid metabolites as previously described by Shideler et al. (1993), with the following

modifications. Briefly, a predetermined mass (0.3 g) of dried, crushed feces was added to 10.00 mL of modified EIA phosphate buffer (0.1 M, pH 7.0, 0.1% BSA with 5.0% Tween 20 and 40.0% methanol) in a preweighed glass test tube. Solubilized samples were shaken at room temperature for 24 hr and then centrifuged at $1,000 \times g$ for 10 min. The liquid portion of the modified phosphate buffer was decanted, the residual disposed of, and the supernatant diluted in distilled water at a ratio of 1:10 for the pregnanediol-3-glucuronide (PdG) EIA and 1:4 for the estrone conjugate (E_1C) EIA. Characterization of the antibodies used have been published elsewhere (Munro et al. 1991).

Fecal Extraction Efficiencies

We conducted fecal extraction tests to re-evaluate the extraction efficiencies of fecal samples specifically for tule elk to which labeled estrone sulfate or progesterone was added. The tracer was added to wet feces prior to drying and solubilization. The resulting supernatant and pellet were extended with scintillation fluid and counted, yielding recoveries of 35% for estrone sulfate and 47% for progesterone metabolites. These recovery values are consistent with those reported by Shideler et al. (1993) for macaque (*Macaca fascicularis*) feces via the same technique (40% for $^3\text{H}\text{-E}_1\text{SO}_4$ and 32% for $^3\text{H}\text{-PdG}$).

Enzyme Immunoassays

We measured concentrations of fecal steroids via EIA techniques described by Shideler et al. (1993) for PdG and E_1C . Diluted samples were added directly to previously antibody-coated 96-well microtiter plates, and samples were run in quadruplicate on the same plate to determine the mean PdG and E_1C concentrations. We added internal controls and standards to each plate to ensure quality control and to construct a standard curve (Shideler et al. 1993). Standard concentrations ranged from 6.25 to 200.00 pg/well for the E_1C EIA, 0.04–5.00 ng/well for the PdG EIA for samples collected during the rutting season, and 0.02–2.50 ng/well for the PdG EIA for samples collected after the breeding season. The interassay coefficients of variation (CVs) for the E_1C EIA were 16.0% at both 72.0 and 41.0% bound ($n = 54$). The 50% binding of the E_1C EIA standard curve averaged 48.30 ± 7.49 pg/well; $\bar{x} \pm \text{SD}$; $n = 54$. The E_1C EIA of serial dilutions of fecal samples from collared

and uncollared cows exhibited a response parallel to the E_1C standard curve. The interassay CVs for the PdG EIA were 12.0% at 47.0% bound, and 14.0% at 22.0% bound ($n = 54$). The 50.0% binding of the PdG EIA standard curve averaged 0.12 ± 0.02 ng/well ($n = 54$). The PdG EIA of serial dilutions of fecal samples from collared and uncollared cows exhibited a response parallel to the PdG standard curve. Serum progesterone concentrations were determined for serum samples collected in November 1996 by the methods of Munro and Stabenfeldt (1984).

Statistical Analysis

Fecal concentrations of PdG and E_1C were recorded by the calendar day of collection for each radiocollared cow. Early (1–3 months), middle (4–6 months), and late pregnancy (7–9 months) concentrations of PdG and E_1C were determined by aligning the highest observed estrogen conjugate concentrations found to occur within the last trimester of gestation. We used standard descriptive statistics to summarize results, and we applied Mann-Whitney rank sum tests (U) to test statistical significance. Based on the hormonal concentrations obtained from pregnant and nonpregnant radiocollared cows from July 1996 through March 1997, confidence limits (95.0%) were established for predicting pregnancy status from hormonal metabolite concentrations obtained for a single fecal sample. All means are reported \pm standard deviation.

RESULTS

Mean fecal progesterone metabolite concentrations in cows that produced calves were 2.05 ± 0.74 ug/g dmf (range = 1.02–4.11) during early pregnancy, 3.39 ± 1.24 ug/g dmf (range = 1.01–9.26) during middle pregnancy, and 3.93 ± 1.36 ug/g dmf (range = 1.38–6.82) during late pregnancy (Fig. 1). Mean progesterone metabolite concentrations in cows that did not produce calves were 1.36 ± 0.71 ug/g dmf (range = 0.49–2.85) during the breeding season and 0.65 ± 0.11 ug/g dmf (range = 0.46–0.85) after the breeding season. Differences in progesterone metabolite concentrations between pregnant and nonpregnant cows were significant following the breeding season ($U = 78.00$, $P < 0.001$). Among the concentrations of PdG excreted during pregnancy, the lowest P -value ($P < 0.001$) was obtained in comparison of early

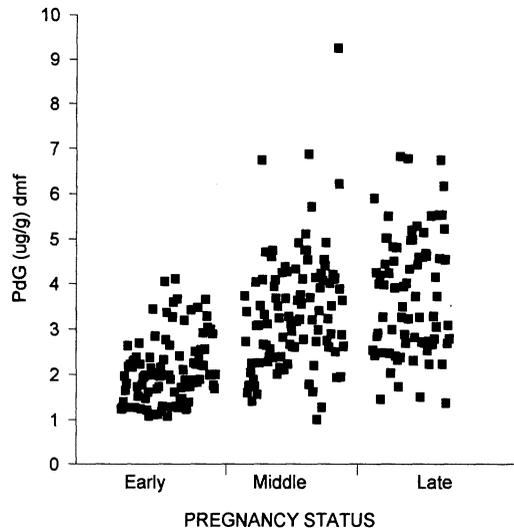


Fig. 1. Concentrations of pregnanediol-3-glucuronide (PdG) excreted by pregnant radiocollared tule elk cows. Early (1–3 months), middle (4–6 months), and late pregnancy (7–9 months) concentrations of PdG in ug/g dry mass feces (dmf) were determined by aligning the highest observed estrogen conjugate concentrations found to occur within the last trimester of gestation.

PdG concentrations with those excreted during both middle ($U = 6612.5$) and late ($U = 9654.0$) pregnancy. The highest P -value ($U = 7821.5$, $P = 0.01$) was obtained in comparison of progesterone metabolite concentrations excreted during middle versus late pregnancy.

Mean fecal E_1C concentrations in cows that produced calves were 72.2 ± 34.1 ng/g dmf (range = 32.2–255.9) during early pregnancy, 138.6 ± 47.6 ng/g dmf (range = 45.7–335.4) during middle pregnancy, and 887.9 ± 491.7 ng/g dmf (range = 205.9–1955.3) during late pregnancy (Fig. 2). Mean E_1C concentrations in cows that did not produce calves were 80.5 ± 41.9 ng/g dmf (range = 32.2–204.5) during the breeding season and 69.9 ± 35.2 ng/g dmf (range = 39.5–144.8) after the breeding season. During early pregnancy, fecal E_1C concentrations were indistinguishable ($U = 3,826.5$; $P = 0.77$) from those excreted in nonpregnant, cycling cows during the breeding season and nonlactating, nonpregnant cows following the breeding season. Significant differences in E_1C concentrations between pregnant and nonpregnant cows occurred during middle ($U = 1,991.0$; $P < 0.001$) and late pregnancy ($U = 1,452.0$; $P < 0.001$). Estrogen conjugate concentrations were found to rise gradually beginning approximately 90 days prior to a sighting

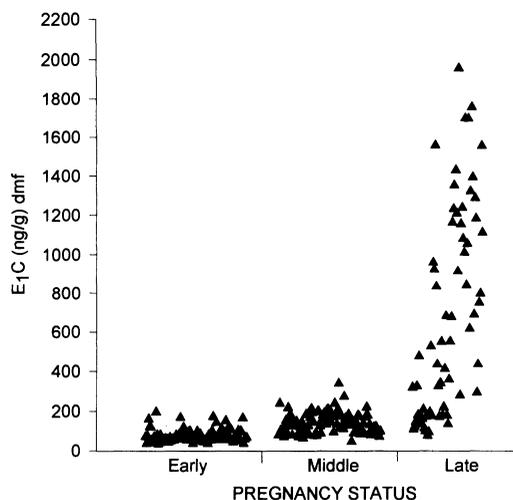


Fig. 2. Concentrations of estrone conjugate (E_1C) excreted by pregnant radiocollared tule elk cows. Early (1–3 months), middle (4–6 months), and late pregnancy (7–9 months) concentrations of E_1C in ng/g dry mass feces (dmf) were determined by aligning the highest observed estrogen conjugate concentrations found to occur within the last trimester of gestation.

of a nursing calf. The highest concentrations of E_1C were excreted approximately 3–30 days prior to sighting of a calf.

Among the 16 radiocollared cows that remained from the 1995 capture and collaring, 16 became pregnant during the 1996 breeding season. One of the cows appeared to have conceived twice and to have lost both pregnancies in the first trimester. The remaining 15 cows had elevated PdG concentrations in fecal samples collected throughout gestation. Significant ($U = 7,834.0$, $P < 0.001$) increases in E_1C concentrations occurred prior to parturition in all but 1 pregnant cow. Of the 14 cows that showed significant increases in E_1C concentrations, all were later sighted nursing a calf. Fecal samples were collected from 9 nursing cows, all of which exhibited PdG and E_1C concentrations similar to those excreted by nonpregnant cows ($U = 177.0$, $P = 0.321$). The 1 cow that did not show an increase in E_1C concentrations did not have a prepartum fecal sample collected during the final 90 days of gestation. However, this cow was later sighted nursing a calf, and a postpartum fecal sample revealed a decline in PdG and E_1C concentrations consistent with nonpregnant levels.

Among the second group of cows ($n = 18$) captured and radiocollared in 1996, 16 became pregnant during the 1996 breeding season. One

of the 16 cows appeared to have aborted 2 months after capture, as evidenced by a decline in consistently high PdG concentrations. The remaining 15 cows had elevated PdG concentrations sustained throughout gestation. Significant increases in E_1C concentrations occurred in 14 of the 15 pregnant cows approximately 3–60 days prior to parturition; a fecal sample was not obtained from 1 cow during the final, approximately 90 days of gestation. Among the 14 cows that exhibited a rise in E_1C concentrations, 10 were later sighted nursing calves. Fecal samples were collected from 8 nursing cows, all of which measured a postpartum decline in PdG and E_1C concentrations consistent with nonpregnant levels. The 1 cow that did not have a prepartum fecal sample was later sighted nursing a calf, and a fecal sample was collected that confirmed E_1C and PdG had returned to nonpregnant concentrations. While 4 pregnant cows were never sighted nursing a calf, fecal samples were collected from each of the cows after their expected calving date. All samples showed a decline in PdG and E_1C concentrations consistent with nonpregnant levels, which served as confirmation that cows were no longer pregnant.

Analysis of serum progesterone obtained at the time of capture from the same 18 cows indicated 17 of the 18 cows had a corpus luteum present at the time of radiocollaring. After the calving season, it was possible to separate the cows into 2 groups: early pregnant cows and nonpregnant cycling cows. Mean serum progesterone concentrations were 3.35 ± 0.46 ng/mL (range = 0.90–7.20) in early pregnant cows and 1.00 ± 0.52 ng/mL (range = 0.10–1.90) in nonpregnant cycling cows. Retrospective analysis revealed 1 cow was misdiagnosed as pregnant based on a serum progesterone concentration of 1.9 ng/mL. A fecal sample collected from the same cow on the same date that serum was obtained indicated this cow was not pregnant based on a fecal PdG concentration of 0.51 μ g/g dmf (Fig. 3). The values for progesterone obtained from the sera of cow tule elk are considered as immunoreactive concentrations, because this assay was not validated specifically for tule elk. However, because this assay has been validated in various domestic animal species (Munro and Stabenfeldt 1984), it is unlikely the values obtained do not accurately reflect concentrations of progesterone in tule elk.

Confidence limits established from the me-

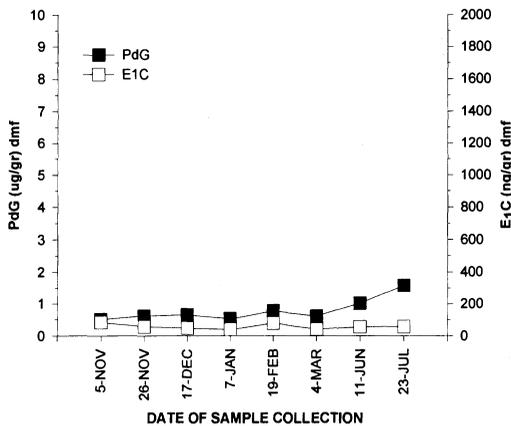


Fig. 3. Longitudinal profile of pregnanediol-3-glucuronide (PdG) and estrone conjugate (E₁C) excretion in a nonpregnant cow tule elk. Each fecal sample is represented by the date and month of collection. This cow was captured and radiocollared in November 1996. The profile reflects the pattern of PdG and E₁C excretion during seasonal anestrus and ovarian recrudescence for the 1997 breeding season. Based on behavioral observations, this cow began to exhibit estrous behavior and started cycling in June 1997 coincident with PdG concentrations >1.01 ug/g dry mass feces (dmf).

tabolite concentrations excreted by radiocollared cows were used for determining the accuracy of pregnancy diagnosis based on a single fecal sample collected from July 1996 through March 1997. Progesterone metabolite (PdG) concentrations were 100% reliable at predicting pregnancy within the first trimester of gestation through parturition in radiocollared cows. Estrogen metabolite (E₁C) concentrations were not a reliable indicator of pregnancy status until the last trimester of gestation. A progesterone metabolite concentration ≥ 1.01 ug/g dmf after the breeding season was set as confirmation of pregnancy status in fecal samples collected from uncollared cows. Based on fecal samples collected from uncollared cows from December 1996 through March 1997, the pregnancy rate was estimated at 64% (133 cows; Fig. 4). In February 1997, the first calf of the 1997 calving season was sighted, and newborn calves were sighted into August 1997. The results of routine calf counts established an early value of 121 calves, while 103 calves were counted at the end of the 1997 calving season (J. A. Howell, USGS-BRD, personal communication).

DISCUSSION

Estrogen conjugate and progesterone metabolite concentrations measured in fecal samples collected from free-ranging cow tule elk provide a noninvasive method for accurately de-

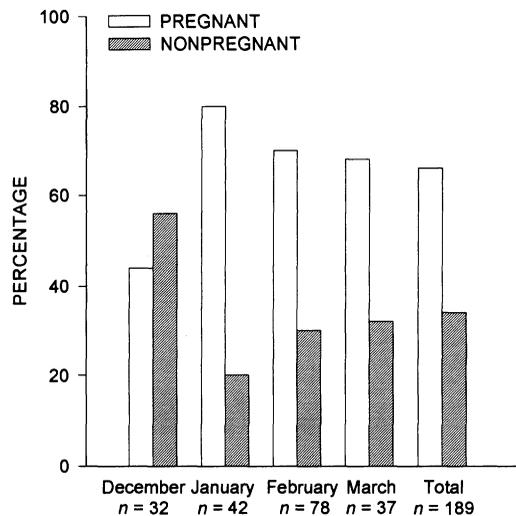


Fig. 4. A summary of fecal samples collected from a population of 208 uncollared tule elk cows. The percentages of uncollared cows in the population diagnosed as pregnant and nonpregnant based on fecal PdG concentration from December 1996 through March 1997 are represented in the bar graph. The estimation of the population pregnancy rate is represented in the last column. Pregnanediol-3-glucuronide (PdG) concentrations were grouped according to month of collection. Beginning in December 1996, PdG concentrations ≥ 1.01 ug/g dry mass feces (dmf) were 100% reliable at predicting pregnancy in radiocollared cows.

tecting and monitoring pregnancy. Longitudinal fecal hormone profiles of pregnant cows allowed determination of the time course and dynamics of PdG and E₁C excretion from conception until calving. The profiles of nonpregnant cows revealed patterns of PdG and E₁C excretion indicative of cyclicity and seasonal anestrus.

Mean fecal PdG concentrations in pregnant and nonpregnant cows were different during and after the breeding season. Following the breeding season, no nonpregnant cow had a PdG concentration higher than or equal to the concentrations excreted by pregnant cows. This finding was not always true during the breeding season, when normal mature cows were cycling. The results of studies of other wild ungulate species have shown that progesterone secretion in nonpregnant females during the breeding season, at the luteal phase of their estrous cycle, can reach concentrations equal to those of early pregnancy (Plotka et al. 1977, Barrell and Bos 1989).

In pregnant cows, PdG concentrations remained elevated throughout gestation. Following the first third of pregnancy, mean PdG concentrations increased and remained elevated until calving. This increase in PdG concentra-

tions probably reflected progesterone production by the placenta. Similar increases in fecal PdG concentrations at midgestation have been documented in the black rhinoceros (*Diceros bicornis michaeli*; Schwarzenberger et al. 1993a) and okapi (*Okapia johnstoni*; Schwarzenberger et al. 1993b). Fecal samples collected from cows that had calved indicated that PdG concentrations declined to nonpregnant concentrations following parturition.

Fecal E₁C concentrations among pregnant and nonpregnant cows were not significantly different during the early stages of pregnancy. During the final third of pregnancy, however, fecal E₁C concentrations increased 5–10-fold over those excreted during both the early and middle stages. This increase in E₁C is consistent with findings from other studies of wild ungulate species such as moose (*Alces alces*; Monfort et al. 1993), Rocky Mountain elk (*Cervus elaphus nelsoni*; White et al. 1995), and caribou (*Rangifer tarandus*; Messier et al. 1990), all of which exhibited a rise in E₁C during the final 30–50 days of gestation. This increase in estrogens is believed to be derived from fetal–maternal biosynthesis and likely is involved in the initiation of parturition (Monfort et al. 1993). Assessing reproductive status in animals with this pattern of E₁C excretion is possible by collecting fecal samples during the later stages of pregnancy because the rise in E₁C serves as an accurate predictor of impending parturition.

Serum progesterone concentrations obtained from the 18 radiocollared cows captured in November 1996 were used to assess pregnancy status. Based on serum progesterone concentrations, 1 cow was incorrectly classified as pregnant. A paired fecal sample indicated her nonpregnancy status based on fecal PdG concentration. The timing of serum collection was at the end of the breeding season, so this cow was possibly still cycling. The serum-based pregnancy diagnosis appears unlikely due to the low PdG concentration obtained from the paired fecal sample and from fecal samples obtained over the 5 months that followed. A possible explanation for the serum-based misdiagnosis is that this cow was stressed during capture, resulting in heightened secretion of adrenal progesterone, which has been shown to confound the reproductive endocrine state in other wild ungulates such as the white-tailed deer (*Odocoileus virginianus borealis*; Wesson

et al. 1979, Plotka et al. 1983) and fallow deer (*Dama dama*; Asher et al. 1989).

Fecal samples collected during this study were coupled with observational data to assess the demographic parameters of calf production and mortality in the radiocollared cows. Any cow that showed a rise in E₁C concentrations but was not sighted with a nursing calf was assumed to have calved. It was further presumed that calf mortality had occurred based on the absence of a calf.

Hormonal concentrations from the samples collected from the radiocollared cows in which pregnancy and outcome were known were used to set hormone concentration levels and confidence limits for pregnancy detection in cross-sectional samples collected from nonradiocollared cows. Progesterone metabolite concentrations were effective at predicting pregnancy with highest accuracy after the breeding season. Prior to December 1996, concentrations of PdG excreted by pregnant cows were found to overlap with the concentrations excreted by nonpregnant cycling cows. Using fecal PdG concentrations obtained from uncollared cows from December 1996 through March 1997, we were able to predict a 64% pregnancy rate. Using results obtained from fecal analysis, we were able to estimate fetal loss (6%) and calf mortality (13%) among the radiocollared cows. Because these cows were representative of cows of reproductive age in the herd, it could be assumed that a similar percentage of uncollared cows experienced calf mortality. The 13% calf mortality based on fecal analysis appears consistent with a 0.85 survivorship as determined by a demographic study (J. A. Howell, USGS-BRD, personal communication). When these factors were considered, the estimate of the pregnancy rate, based on fecal metabolite concentrations, was highly accurate in comparison to the observed calving rate of 58% (121 calves). While it may appear that the estimated pregnancy rate of 64% is low, the total number of cows during this study was 208, with approximately 135 being ≥3 years old (J. A. Howell, USGS-BRD, personal communication). There is little evidence that young cows (1–2 yr old) are breeding (J. A. Howell, USGS-BRD, personal communication). The pregnancy rate for all cows was 64% (133 of 208 cows), while the pregnancy rate of cows considered of reproductive age would average 98% (133/135). By including younger cows (1–2 yr old), which are generally not considered

reproductively active, the pregnancy rate becomes 64%. Furthermore, the calving rate of 58% considers the total number of cows in the herd ($n = 208$). Early calf counts showed 121 calves, while the number dropped to 103 at the end of the calving season. If one examines the calving rate of cows of reproductive age (≥ 3 yr old; $n = 135$), it averages 89.6% (121/135). By including the younger cows (1–2 yr old), which are generally not considered reproductively active, the calving rate becomes 58%.

White et al. (1995) and Garrott et al. (1998) have also examined the ability to diagnosis pregnancy in free-ranging Rocky Mountain elk, but they used different assay techniques. Using enzyme immunoassay, White et al. (1995) was able to measure free progesterone (P_4), PdG, and E_1C from fecal samples collected from pregnant and nonpregnant cow elk following the breeding season. They determined that concentrations of P_4 and PdG measured in multiple fecal samples collected during late gestation was most accurate at predicting pregnancy status. Garrott et al. (1998) used a radioimmunoassay technique to validate single sample pregnancy diagnosis based on fecal progestagen (P_4) concentration obtained from samples collected following the breeding season.

MANAGEMENT IMPLICATIONS

The present study combines data from a longitudinal study of individually identified animals (radiocollared cows) and concurrent data from a cross-sectional study of unidentified animals (uncollared cows) to illustrate the value of fecal steroid measurements in assessing reproductive status in both groups. Results from the longitudinal sampling of identified animals demonstrate the feasibility of collecting serial samples from specific, free-ranging animals, and the ability to remotely detect a series of reproductive events in a subpopulation. Because the breeding season of tule elk at Point Reyes National Seashore can be relatively asynchronous, this part of the study was critical. The longitudinal sampling permitted the hormonal data to be aligned with the calving event such that the hormonal dynamics associated with breedings and abortions could be detected. For example, the pregnancy losses and the late pregnancy rise of fecal estrogens (which appears to be a specific marker for impending parturition) would not have been identified without the longitudinal component. Results from the cross-sectional

study provide proof of the general concept that fecal steroids can be used for assessing pregnancy rates and estimating or predicting population increase in free-ranging populations. Using validation of the general concept here, biologists can conduct future assessments of tule elk via population-based samples only.

In conclusion, fecal steroid assessments of reproductive status provide an important method with which to evaluate the reproductive success of wildlife populations. In conjunction with mathematical modeling, fecal steroid measurements could provide a point estimate of fecundity from a single sampling. Development of such a model that uses an actual rather than a theorized estimate of reproductive potential provides a useful tool in the management of wildlife populations as well as in the study of factors that influence their reproductive success.

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