Determining Pregnancy Status Of Rocky Mountain Bighorn Ewes From Fecal P4, A Progesterone Derivative Hormone.

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Abstract: The pregnancy status of ten Rocky Mountain bighorn ewes (*Ovis canadensis canadensis*) was evaluated using fecal P4, a progesterone derivative hormone. Six fecal samples from each individual were collected during the period of time corresponding to the second trimester of pregnancy and measured for P4. Mean values of each ewe were compared to 95% confidence intervals from P4 values of 23 samples collected during the same time period from known pregnant ewes. Eight of the ten ewes tested were not pregnant during this time period and the pregnancy status of the other two ewes was inconclusive. This non-invasive technique for assessing pregnancy status has a great potential for gathering information without causing any stress to the animal, and a more rigorous study to validate this technique is currently underway.

INTRODUCTION

The use of non-invasive techniques for evaluating pregnancy status of wild ungulates is becoming more common. Evaluation of various fecal metabolites, including estrone conjugates (E_1C), pregnanediol-3-glucuronide (IPdG), and free progesterone (P_4), has been preformed in ungulates such as moose (*Alces alces*) (Monfort et al. 1993), caribou (*Rangifer tarandus*) (Messier et al. 1990), elk (*Cervus elaphus*) (Garrott et al. 1998, White et al. 1995), and bighorn sheep (*Ovis canadensis*) (Borjesson et al. 1996).

Elevated P_4 is observed during the estrous cycle, and can be used to monitor pregnancy status since it increases throughout pregnancy. There is an abrupt decline in P_4 concentration after parturition, and a drop during pregnancy is an indication of fetal loss (Cook et al.2001). Both radio immunoassay (RIA) and enzyme immunoassay (EIA) can be used to detect P_4 levels. EIA is less expensive, but may require a larger number of samples to accurately evaluate pregnancy status (Garrott et al. 1998).

In an effort to explore the validity of using fecal P_4 to assess pregnancy status in Rocky Mountain bighorn sheep (*O. c. canadensis*), P_4 concentrations were analyzed in fecal samples collected from known pregnant and known nonpregnant ewes in Custer State Park, SD. High natality rates are usually observed in bighorn sheep (Goldstein 2001, Merwin 2000, Brundige 1985, Woodgerd 1964,), but low natality was observed in one subherd of bighorn in CSP during 2000. We used fecal P_4 to evaluate the pregnancy status of 10 Rocky Mountain bighorn ewes never observed with a lamb.

STUDY AREA

This study was conducted in Custer State Park in the southeast corner of the

Black Hills, South Dakota. Bighorn sheep in this 29,150 ha park live in four geographically separate subherds. This study focused on two subherds, one in the east end (EE), and one in the west end (WE), of French Creek Canyon. EE and WE bighorns are separate subherds, but have occasional contact where their ranges overlap.

Dominant grasses along this canyon include western wheatgrass (*Pascopyrum smithii*), blue grama (*Bouteloua gracilis*), little bluestem (*Schizachyrium scoparium*), and buffalo grass (*Buchloe dactyloides*) (Morgan 1987, Turner 1974). Canyon rims in EE are characterized by open meadows, and canyon walls have large cliff faces interspersed with ponderosa pine (*Pinus ponderosa*) forests. Attributes of WE are similar, although there are fewer meadows and forests tend to be denser. North and west of French Creek, wildfires burned approximately 8,500 ha in 1988 and 1991. These open hills, rising from approximately 1,250 m to 1,850 m now contain many charred snags and burnt downed woody material.

Other animals living in this study area include mountain lions, coyotes, bison (*Bison bison*), elk (*Cervus elaphus*), white-tailed deer (*Odocoileus virginianus*), mule deer (*O. hemionus*), pronghorn (*Antilocapra americana)*, and a variety of small mammals, herpetiles, songbirds, and raptors.

METHODS

Thirty-two adult bighorn ewes were radiocollared or otherwise uniquely identifiable, and monitored daily for two years between 1999-2000. In 2000, fecal samples from ten marked ewes in the East End subherd in Custer State Park, South Dakota, never observed with a lamb were analyzed for pregnancy status. Six fecal samples from each of these ewes collected between March 1, 2000, and May 14, 2000 were evaluated for P_4, (St. Louis Zoo Endocrinology Laboratory, St. Louis, Missouri, USA). P_4 laboratory extraction methods followed those of Shideler et al. (1993), and radio immunoassay methods followed those of Bauman and Hardin (1998) using reagents from DSL, Webster TX. To evaluate efficacy of P_4 to indicate pregnancy status, these values were compared to P_4 values of 23 samples from known non-pregnant ewes collected from August 1-7, 2000, and to values of 30 samples from known pregnant ewes collected from March 4 – May 3, 2000. Ewes were classified as non-pregnant in August, or as pregnant during spring if they were known to have given birth during the following summer.

ANALYSIS

Mean and 95% confidence intervals (CI) for P_4 values from known pregnant ewes during March and April, and from known non-pregnant ewes during August were calculated. P_4 values for known pregnant ewes were then separated into samples from March, and samples from April, and mean P_4 and 95% CI were calculated for each month. A two-sample t-test was used to compare P_4 values during these two time periods. Mean P_4 values were calculated for each of ten ewes of unknown pregnancy status. These values were not divided by month due to small sample size. These values were compared with 95% CI for P_4 values from known pregnant ewes during March, April, and May combined, and with 95% CI for P_4 values from known non-pregnant ewes, to ascertain pregnancy status

For pregnancy tests evaluating P_4 in only one fecal sample, the lower 95% confidence limit for known pregnant ewes was used as a cutoff value for concluding

Table 1. P_4 values of pregnant versus non-pregnant ewes during different time periods

Pregnancy status	**Mean P_4 (ng/g)**	**95% Confidence interval of the mean (ng/g)**
Non-pregnant Ewes	570.2	354.9-794.5
Pregnant Ewes (March – early May)	1699.1	1316.5-2081.6
Pregnant Ewes, March	1203.8	895.8-1511.8
Pregnant Ewes, April	1990.3	1374.4-2606.2

that a ewe was pregnant. The upper 95% confidence limit for known non-pregnant ewes was used as a cutoff value for concluding that a ewe was not pregnant. Any value falling between these two numbers was regarded as inconclusive.

The number of fecal samples needed to accurately predict pregnancy status was calculated by selecting one random sample from a given known pregnant ewe. If this value predicted pregnancy, then only one sample was needed for the test. If not, a second sample was randomly selected and this value was averaged with the first (samples were chosen without replacement). This process was repeated until the average of the samples exceeded the lower 95% confidence limit for pregnancy.

RESULTS

Mean and 95% CI for pregnant and non-pregnant ewes are reported in Table 1, as are values for pregnant ewes during March and during April. Mean P_4 values from pregnant ewes were higher in March than they were in April (*P*=0.04). Mean P_4 values for the 10 ewes of unknown pregnancy status are presented in Table 2. Eight of ten ewes tested for pregnancy status were not pregnant during the time period corresponding with the second and the beginning of the third trimesters of pregnancy (March-May 2000). Pregnancy status of two ewes could not be determined with certainty because their average P_4 values fell between the upper 95% confidence limit for non-pregnant ewes, and the 95% confidence limit for pregnant ewes.

Results of using the P_4 value from one fecal sample and comparing it with the upper 95% confidence limit for non-pregnant ewes and with the lower 95% confidence limit for pregnant ewes to predict pregnancy status are presented in Table 3. The numbers of pellets groups needed to accurately predict pregnancy status are presented in Table 4.

DISCUSSION

The use of noninvasive techniques to obtain biological samples is generally less expensive, logistically easier, and less stressful to the animal than the use of invasive techniques. Observing a minimum of eight of 10 ewes tested not being pregnant is quite rare in bighorn sheep. Among marked ewes in the East End subherd, 19 of 20 were observed with lambs in 1999, compared with 4 of 13 in 2000. Two additional marked ewes disappeared during early summer 2002 before the peak of lambing (and thus before they had the opportunity to be observed with a lamb), but only one of these ewes was analyzed for pregnancy status in this study.

In a second subherd in CSP (West End), 8 of 8 marked ewes were observed with a lamb in 1999, and 10 of 11marked ewes were observed with a lamb in 2000.

Table 2. Pregnancy status during the second trimester of pregnancy 2000 (March-April) of marked ewes in East End never observed with a lamb, compared with 95% confidence intervals of mean values form pregnant ewes, and from nonpregnant ewes. Values exceeding 1316.5 ng/g indicate pregnant and values under 794.5 ng/g indicate not pregnant.

ID	P4 averages	Pregnant?
29g	680.6	NO
2y	1067.8	UNKNOWN
34g	161.4	NO
47g	279.8	NO
Black	502.1	NO
EE black red	679.3	NO
Green	449.9	NO
Green red	662.6	NO
Lf horn II	549.4	NO
Orange	800.6	UNKNOWN

Table 3. Predicted pregnancy status when comparing the P_4 value of one fecal sample from a ewe of known pregnancy status with a reference group of a larger number of samples from ewes of known pregnancy status.

	Sample size (number of fecal samples comprising the reference group)	Number of incorrect results using a single fecal sample	Number of ambiguous results using a single fecal sample	Number of correct results using a single fecal sample
Non-pregnant	23	2 (9%)	2 (9%)	19 (82%)
Pregnant (March and April samples)	30	3 (10%)	9 (30%)	18 (60%)
Pregnant (March samples only)	14	3 (22%)	1 (7%)	10 (71%)
Pregnant (April samples only)	17	1 (6%)	5 (30%)	11 (64%)

Whether the 10 ewes never observed with a lamb aborted during the time period corresponding with the first trimester of pregnancy, or were never bred cannot be concluded from this data, but either case is unusual.

Of the two ewes whose pregnancy status could not be ascertained, the average P_4 value of one of these ewes (Orange) was very close to the 95% confidence limit of non-pregnant ewes, and there was no trend in the values to suggest either pregnancy or an abortion. She was likely not pregnant. The other ewe (2y) had low P_4 values in March, corresponding with non-pregnant status, but high levels in April, corresponding with pregnant status. Had this ewe been bred, she would have been a yearling at the time. It is uncommon, but not unheard of, for two-year olds to lamb. In addition, this ewe was never observed with a

Table 4. Number of pellet groups per individual needed to accurately predict pregnancy status. All ewes presented in the table were known pregnant ewes.

	March	April	March-April
Blue black	2	2	1
Black red	1	1	1
Blue green	1	1	1
Brown yellow	1	1	1
Green yellow	2	1	4
red	(only one sample available for comparison)	2	3
Average	1.4	1.3	1.8

swollen udder. It is possible that she was bred late, therefore her P_4 levels did not rise perceptibly until April, and then aborted, but the results remain inconclusive.

Results from this pilot study indicate that radioimmunoassay of fecal P_4 may provide a reliable, noninvasive technique for assessing pregnancy status in bighorn sheep. One potential problem with this study is that samples from known pregnant ewes were collected from March through May, whereas samples from known nonpregnant ewes were collected during August. The most accurate comparison would be among fecal samples collected during the same time period. However, samples were collected from wild bighorns and it is not possible to differentiate between ewes which were not bred and those that lost a lamb prior to observation (either pre- or post-natal). Such samples could only come from a captive herd. Bjoresson et al. (1996) found that IPdG, a metabolite of P_4, concentrations in nonpregnant bighorn did not increase between November and June. P_4 concentrations should follow the same trend as IPdG for non-pregnant ewes, therefore P_4 values of non-pregnant ewes should not be different during March – May than during August.

Bighorn breeding peaks in December in CSP (Brundige et al. 1988). Ewes that are not bred in December may experience a second, and potentially a third estrous in January and February, respectively. Elevated P_4 levels during estrous may be erroneously interpreted as pregnancy, therefore samples were not collected prior to March.

Samples collected from pregnant ewes in March only, April only, and March and April combined, yielded accurate results 71%, 64%, and 60% of the time, respectively, when using a single fecal sample to predict pregnancy status. Small samples sizes and differences in sample sizes for the reference group may have influenced the results, making it difficult to conclude which collection period would yield the most accurate results. However, March and April combined had the largest sample size but the lowest degree of accuracy, therefore choosing a single month should yield more accurate results. There was greater variation in samples collected in April compared with March and with March and April combined. This may be due in part to two outlier values occurring during April, but none occurring during March. Increased variation may be a function of increased P_4 values during this time period. Borjesson et al. (1996) found the standard deviation of IpdG levels for bighorn sheep increased from 0.47 during 0-60 days of pregnancy, to 1.18 during 60-180 days of pregnancy.

Both iPdG and P_4 concentrations were demonstrated to be higher during late gestation than during early gestation in elk (White et al. 1995), and that held true for iPdG (Bjoresson et al. 1996) and P_4 in bighorn sheep during this study. Misdiagnosis of pregnancy happened most often during March, presumably because P_4 values in March are closer to P_4 values of non-pregnant ewes than they are in April. Based on this parameter, it may be more desirable to collect samples as late during pregnancy as possible. Given the small overlap of P_4 values of pregnant and non-pregnant ewes, it may be necessary to use more than one fecal sample to increase accuracy of the results. Four samples always predicted accurate results, regardless of the time period. However, two samples always predicted accurate results when sampling from March only or April only. Therefore, it would be more accurate to restrict sample collection to as narrow a time frame as possible.

Differences in P_4 values between non-pregnant and pregnant ewes, and pregnant ewes during the second and beginning of the third trimesters of pregnancy are large enough with small enough variation to be a useful technique in assessing pregnancy status. A minimum of two samples collected within the same month as late during the pregnancy cycle as possible is recommended to increase accuracy of the results. The small sample size used (n=6 ewes) during this study inhibits a more in depth evaluation of collecting samples during March versus April, and sampling during a shorter time period. A study using a larger sample size is currently underway to validate this technique for bighorn sheep, and assess how many samples per ewe should be analyzed.

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