DOI: 10.7589/JWD-D-22-00041
Journal of Wildlife Diseases, 59(1), 2023, pp. 61–70
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EVALUATING THE EFFICACY OF NONINVASIVE FECAL SAMPLING FOR PREGNANCY DETECTION IN ELK (*CERVUS CANADENSIS*)

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ABSTRACT: Elk (*Cervus canadensis*) were reintroduced to Tennessee, USA in the early 2000s, with limited reproductive monitoring since initial release. We assessed the efficacy of noninvasive sampling for determining pregnancy using invasive (capture) and noninvasive (fecal collection in the field) techniques at the North Cumberland Wildlife Management Area (NCWMA), Tennessee. We captured 20 female elk 2019–2020, used pregnancy-specific protein B (PSPB) in blood to determine pregnancy and compared results to fecal progesterone metabolite (FPM) concentrations using two commercially available enzyme immunoassay (EIA) kits. Based on PSPB concentrations, 8/11 and 3/4 of captured adult elk (≥2.5 yr of age) were pregnant in 2019 and 2020, respectively; no 1.5-yr-old elk were pregnant (n=5). Using the progesterone EIA kit, FPM concentrations were $\bar{x}$=192.84±38.63 ng/g (95% CI, 96.48–289.20) for nonpregnant and $\bar{x}$=536.17±74.98 ng/g (95% CI, 375.97–696.36) for pregnant captured females. For the progesterone metabolite kit, FPM concentrations were $\bar{x}$=188.16±43.39 ng/g (95% confidence interval [CI], 76.63–299.69) for nonpregnant and $\bar{x}$=693.52±126.52 ng/g (95% CI, 407.31–979.72) for pregnant captured females. From February to May 2019, we collected 357 fecal samples in 65 areas across 489.62 km^2 of the NCWMA. Using extracted DNA and analysis of 15 microsatellites, we identified 62 unique individuals from 128 female fecal samples collected on the landscape. We categorized females from landscape-collected feces as nonpregnant (35.5–40.3%; Metabolite-EIA kits), undetermined (1.6–6.5%; Metabolite-EIA kits), or pregnant (62.9–53.2%; Metabolite-EIA kits) based on a 95% CI of captured female FPM concentrations, giving an overall pregnancy rate of 53.2% using the recommended EIA kit. The pregnancy rate in sexually mature females may be higher, as it was not possible to distinguish age classes of landscape-collected fecal samples; therefore, some may have been from younger age classes not expected to be pregnant. Analysis of FPM may be useful at a population level to detect pregnancy.

Key words: *Cervus canadensis*, elk, fecal progesterone metabolites, noninvasive techniques, population, pregnancy, progesterone.

INTRODUCTION

Elk were reintroduced to the North Cumberland Wildlife Management Area (NCWMA), Tennessee, USA beginning in the early 2000s and have since established a population in east Tennessee. Detailed information on the reintroduction was described by Kindall et al. (2011).

Determining pregnancy is important for evaluating the health and growth potential of wild animal populations. The pregnancy status of adult females released during the initial 3 yr of reintroduction was determined as 57.7, 73.7, and 93.3%, respectively (Muller et al. 2004). Pregnancy status has not been reported for the population since then; updated information on elk pregnancy at the NCWMA is needed to assess population growth potential.

Pregnancy-specific protein B (PSPB), a protein hormone secreted by the placenta in ruminants, may be used to determine pregnancy with high accuracy in many species, including elk (*Cervus canadensis*; Noyes et al. 1997; Seixas et al. 2019) and white-tailed deer (*Odocoileus virginianus*; Osborn et al. 1995). However, PSPB must be measured from a blood sample, requiring capture. Thus, PSPB

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testing for large-scale population evaluation may be difficult and impractical.

Reliable methods to measure reproductive steroid hormone concentrations also enable monitoring individuals and reproductive efforts (Graham et al. 2001; Kersey and Dehnhard 2014). Progesterone is a progestin steroid produced by the corpus luteum after ovulation, the corpus luteum and placenta during pregnancy, and the adrenal gland (Plotka et al. 1982; Taraborrelli 2015; Litwack 2018). In elk, progesterone levels increase after conception and remain high throughout gestation before declining shortly before parturition (Hudson and Haigh 2002). The most accurate method for measuring true concentrations of circulating steroid hormones is through a blood sample (Touma and Palme 2005; Peter et al. 2018), but stress during capture may affect serum progesterone concentrations and may lead to misidentification of pregnancy status (Hollenstein et al. 2006; Togashi et al. 2009). For example, in white-tailed deer, high concentrations of serum progesterone were found to be excreted by the adrenal gland during capture events (Plotka et al. 1982).

Measuring serum hormone concentrations on large spatial and temporal scales from wild, free-ranging animals is not always feasible (Borque et al. 2011). Invasive methods require chemical immobilization or physical restraint of an animal, which may influence circulating hormone concentrations (Messier et al. 1990; Cain et al. 2012). Steroid hormones, which are predominately metabolized by the liver and excreted in urine or feces, may be quantified via metabolites (Schwarzenberger et al. 1996). Feces are easily obtained and may be collected across a landscape or gestational period. Steroid metabolites are relatively stable in feces, enable more-frequent collections of samples from the landscape, and are unaffected by invasive sampling stress (Kumar et al. 2013). Fecal progesterone metabolites (FPM) concentrations show similar patterns to plasma values and have been used to monitor pregnancy (Schwarzenberger et al. 1997). Noninvasive techniques to assess fecal progestin, free progesterone, or progesterone metabolite concentrations for determining pregnancy status have been previously evaluated and validated in elk (Garrott et al. 1995; White et al. 1995) and other ungulate species (Morden et al. 2011; Cain et al. 2012; Krepschi et al. 2013; Wang et al. 2016).

The objectives of our study were to compare FPM concentrations of feces from female elk collected at the time of capture with PSPB (standard test for pregnancy determination), compare two commercial progesterone enzyme immunoassay (EIA) kits for FPM analysis, and evaluate the efficacy of noninvasive fecal sampling for determining pregnancy status on a population scale, potentially providing managers with a valuable method for assessing population pregnancy rates.

MATERIALS AND METHODS

Study area

In 2000, Tennessee Wildlife Resources Agency (TWRA) established a 271,139-ha Elk Restoration Zone for elk reintroduction (TWRA 2000). We conducted research at the NCWMA located within the Elk Restoration Zone. The NCWMA is a mixed-mesophytic forest comprised of 79,318 ha of public and leased private land managed by TWRA (TWRA 2018). The NCWMA is approximately 86% deciduous forest, 12% wildlife openings (grasslands, fields, and reclaimed coal strip mines), and 1% cropland (TWRA 2018).

Elk capture and sample collection

All research was approved by the University of Tennessee Institutional Animal Care and Use Committee (UT-IACUC 2671-0219). During the winters of 2019 and 2020, we captured 29 elk (21 females and eight males) at the NCWMA; some elk were caught in corral traps before darting. All elk were anesthetized with an intramuscular injection of 2 mL (females and small males) and 3 mL (large males) of BAM (27.3 mg/mL butorphanol, 9.1 mg/mL azaperone, and 10.9 mg/mL medetomidine; ZooPharm, Windsor, Colorado, USA) administered by 2-mL or 3-mL darts (PneuDart Inc., Williamsport, Pennsylvania, USA) delivered using a Dan-Inject CO2 Rifle (Dan-Inject, Kolding, Denmark). Elk were continuously monitored under anesthesia and vital signs including heart rate, respiratory rate, rectal temperature, and oxygen saturation were record-

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ed every 5–10 min. Oxygen saturation levels were monitored using a hand-held pulse oximeter (PM-60Vet, Shenzhen Mindray Bio Medical Electronics, Shenzhen, China) attached to the tongue. Supplemental oxygen was administered intranasally if oxygen saturation fell below 90%. We recorded sex and age and fitted elk with global positioning system (GPS) collars for concurrent studies. From each elk we collected whole blood from the jugular or cephalic vein using an 18- or 20-gauge needle into 10-mL serum vacutainers with no anticoagulant (BD, Franklin Lakes, New Jersey, USA), hair pulled from the rump, and a rectal fecal sample. We stored blood samples at –20 C until samples were sent off for analysis. We removed vacutainers from the freezer, pulled liquid from clotted whole blood, and shipped samples in 2-mL increments in microcentrifuge tubes. We reversed elk immobilization with an intramuscular injection of 4 mL (females and small males) and 6 mL (large males) of atipamezole (25 mg/mL; ZooPharm) and 0.5 mL (both males and females) of naltrexone (50 mg/mL; ZooPharm) and monitored elk until fully recovered.

Collection of feces from the landscape

Over a 10-wk period from February to April 2019, we collected fecal samples within 65 areas mainly comprised of wildlife openings and fields across 489.62 km^2 of the NCWMA. We searched each area using a transect approximately 3,378 m long and 2 m wide. Areas smaller than the transect distance were searched in their entirety. We established a 1-wk resting period between sampling events to allow time for fresh samples to reaccumulate. To minimize the potential for elk DNA and sample degradation, we waited approximately 1 day after heavy precipitation events before sampling designated collection areas (Brinkman et al. 2009). Based on sampling protocols in similar species, we recorded sample freshness by rating feces on a scale of one to five (one being oldest and five being freshest) and collected samples with a rating four or five (Kirchhoff and Larsen 1998; Lupardus et al. 2011; Goode et al. 2014). We placed 10–15 fecal pellets in a bag and stored samples in a freezer (–20 C) until analysis.

Genetic identification and sex determination

Fecal pellets were allowed to thaw at room temperature for 15 min. We lightly rubbed the outer portions of four partially thawed pellets with a flat toothpick; these were then stored in a paper coin envelope. Similarly to Muller et al. (2018), we sent hair from captured individuals and three toothpicks per fecal sample (landscape samples) to Wildlife Genetics International (WGI; Nelson, British Columbia, Canada) for DNA analysis of 16 microsatellites and a sex marker for individual elk identification. Personnel at WGI analyzed samples using techniques outlined in Muller et al. (2018).

Blood sample PSPB analysis

To determine pregnancy status of captured females, we submitted 2 mL of whole blood to Herd Health Diagnostics (Pullman, Washington, USA) for PSPB testing using the BioPRYN Wild test (BioTracking, Moscow, Idaho, USA). This measures optical density values correlated to the amount of PSPB in a sample to differentiate pregnant and nonpregnant individuals and is an accurate, reliable test for determining pregnancy status in animals $\geq$40 days postmating (Noyes et al. 1997). We calculated the percent of pregnant females out of the total number of captured females for 2019 and 2020.

Fecal sample enzyme immunoassay (EIA)

We evaluated FPM concentration in fecal samples using EIA. Samples were dried for 24 hr at 50 C (Yamato Constant Temperature Oven DKN900, Orangeburg, New York, USA), ground using a mortar and pestle, and 0.2 g of dried, ground fecal material weighed out for extraction. Progesterone metabolites were extracted from weighed samples using 200-proof (absolute) ethanol (Fisher Scientific, Hampton, New Hampshire, USA) and stored following the steroid solid extraction protocol recommended by Arbor Assays (Ann Arbor, Michigan, USA; Arbor Assays 2022). We added 1 mL ethanol for every 0.1 g fecal material, placed tubes on a rocking shaker for 30 min at room temperature, and centrifuged tubes for 16 min at 1,792 $\times$ G. We removed and stored the supernatant in 2-mL microcentrifuge tubes and assayed samples immediately or stored them for up to 30 days at –20 C until analysis. Samples not assayed using both kits within 30 days of initial extraction were discarded and new fecal material was extracted for the assay (Arbor Assays 2022).

We used two commercial EIA kits including Progesterone EIA (K025-H5; Arbor Assays) and Progesterone Metabolite (K068-H5; Arbor Assays) to quantify FPM concentrations in extracted fecal samples. We followed kit protocols and read results at a wavelength of 450 nm using an iMark microplate absorbance reader (Bio-Rad, Hercules, California, USA). All samples and controls were run in duplicate. We conducted a test of parallelism for both kits by serially diluting fecal extract in assay buffer to produce six dilutions (1:50–1:150 range for Progesterone EIA kit, 1:20–

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1:640 range for Progesterone Metabolite kit). We compared the linear portions of the different diluted extracts of unknown samples with the progesterone standard binding curve for equality of slope (Ajó et al. 2022).

To assess any potential matrix interference within both kits, we calculated extraction efficiency of known amounts of progesterone in spiked fecal samples. For the spiked sample, we added 10 µL of 1,000 ng/mL progesterone standard for the Progesterone EIA kit and 15 µL of 1,000 ng/mL progesterone, standard for the Progesterone Metabolite kit prior to extraction. Additionally, we prepared an unspiked sample with an amount of kit assay buffer equivalent to the amount of progesterone added to the spiked sample. Extraction efficiency was calculated by determining the difference between the spiked and unspiked sample concentrations and dividing by the concentration of the spiked sample.

We calculated interassay coefficient of variation (%CV) using the average concentration of one sample run in duplicate on all Progesterone EIA kit microplates (n=19) and Progesterone Metabolite kit microplates (n=22). We divided the standard deviation by the average FPM concentration of the sample and multiplied by 100. We calculated intra-assay %CV for each kit by averaging the %CV for each sample run on all Progesterone EIA kit microplates (n=19) and Progesterone Metabolite kit microplates (n=22) and reported an overall mean for each kit. We excluded and re-ran samples if the duplicate measures had a %CV≥20 and/or specific binding outside the range of 20–80%. Concentration results are expressed as nanograms of hormone per gram of fecal powder (ng/g).

Statistical analysis: We performed statistical analysis in RStudio (R Core Team 2021). We used a Welch Two Sample t-test to compare nonpregnant and pregnant FPM concentrations of captured females by assay kit. The 95% CI from nonpregnant and pregnant captured females with both a blood and fecal sample were used to established FPM concentration reference ranges. We used the determined reference range to categorize the pregnancy status of female fecal samples collected noninvasively. We used the Fisher exact test to evaluate whether pregnancy categorization of samples differed between the two kits. We used a linear mixed model for both the Progesterone EIA kit and the Progesterone Metabolite kit in the package lme4 (Bates et al. 2015). Models assessed how FPM concentrations of females with multiple samples collected changed over time. Individuals were assigned as a random effect to account for repeated samples collected across the sampling period and fixed effects included date and pregnancy status. To meet normality assumptions, we log transformed FPM data. We obtained P-values for the fixed-effects using analysis of variance (ANOVA). We designated statistical significance at $P<0.05$.

RESULTS

We collected 29 hair samples from captured elk and 357 fecal samples from the landscape for genetic analysis. We successfully identified individual genotype from all hair samples and 157 landscape fecal samples (fecal genotyping success rate of 44%). We captured 21 female elk (16 adults and five yearlings), collected a blood sample from 20 females, and collected a fecal sample from 17 females. From noninvasive fecal sampling we identified 128 female samples (62 unique individuals).

Diluted sample progesterone metabolite concentrations for both kits were parallel to the standard curve (Ajó et al. 2022). The intra-assay %CV was 3.1% (n=19) for the Progesterone EIA kit and 5.1% (n=22) for the Progesterone Metabolite kit. The interassay %CV was 9.8% (n=19 microplates) for the Progesterone EIA kit and 16.3% (n=22 microplates) for the Progesterone Metabolite kit. Extraction efficiency was 93% for the Progesterone EIA kit and 85% for the Progesterone Metabolite kit.

Using PSPB from blood (n=20; 15 adults and five yearlings), we categorized 77% (8/11) adults in 2019 and 75% (3/4) adults in 2020 as pregnant. No yearling females (n=5) were pregnant. We used 16 of the 21 captured females (12 adults and four yearlings) with both a blood and fecal sample collected at the time of capture to establish FPM concentration reference ranges for nonpregnant and pregnant females. Using the Welch two-sample t-test, we found a difference (P=0.0009) between nonpregnant ($\bar{x}$=192.84 ±38.63 ng/g, 95% CI, 96.48–289.20) and pregnant ($\bar{x}$=536.17±74.98 ng/g, 95% CI, 375.97–696.36) captured female FPM concentrations with the Progesterone EIA kit (Fig. 1). For the Progesterone Metabolite kit we found a difference (P=0.0008) between nonpregnant ($\bar{x}$=188.16±43.39 ng/g, 95% CI,

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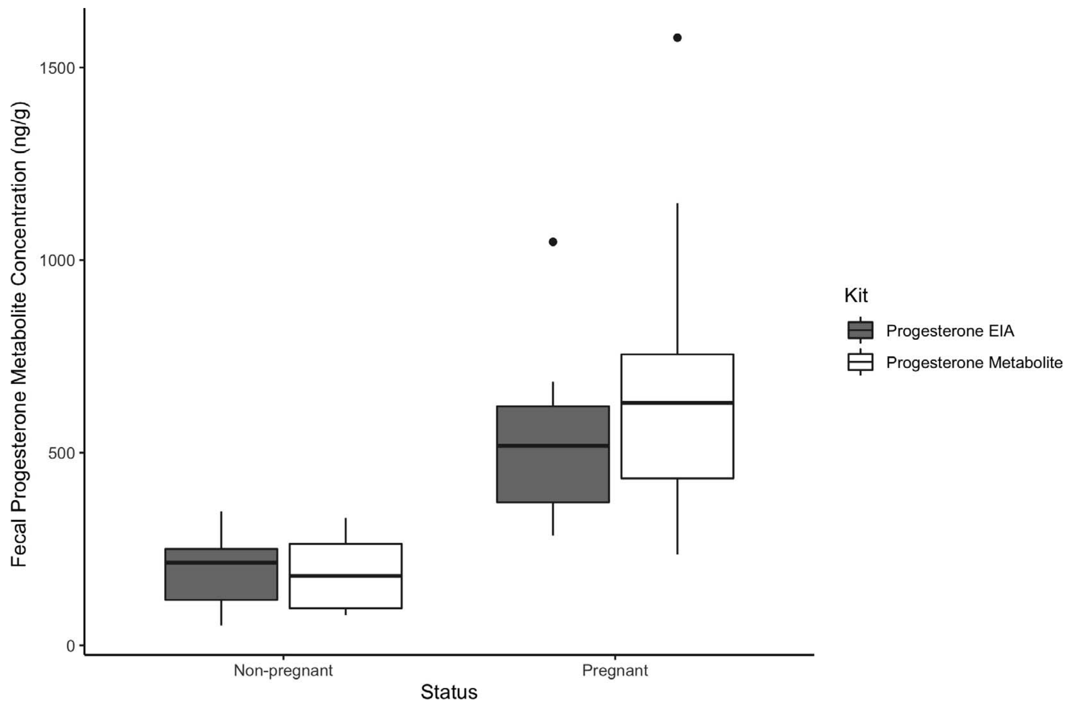

FIGURE 1. Boxplot of the mean fecal progesterone metabolite concentrations of nonpregnant and pregnant female elk (n=16) captured at the North Cumberland Wildlife Management Area in Tennessee, USA (2019–2020) using the Progesterone EIA kit and Progesterone Metabolite kit (Arbor Assays). Pregnancy was determined based on Pregnancy-specific protein B data. Grey boxes represent the Progesterone EIA kit and white boxes represent the Progesterone Metabolite kit.

76.63–299.69) and pregnant ($\bar{x}$=693.52±26.52 ng/g, 95% CI, 407.31–979.72) FPM concentrations (Fig. 1). There was no overlap identified between the 95% CI for nonpregnant and pregnant females, indicating a distinction between FPM concentrations of the two groups. Therefore, we used FPM concentration values of the 95% CI for nonpregnant and pregnant groups to categorize pregnancy status for unknown fecal samples. We classified fecal samples as undetermined when FPM concentrations were between the 95% CI ranges. Using the Progesterone EIA kit, we categorized 25 females as nonpregnant, four as undetermined, and 33 as pregnant (Table 1). Using the Progesterone Metabolite kit, we categorized 22 females as nonpregnant, one as undetermined, and 39 as pregnant (Table 1). We included samples from seven of eight males in the FPM analysis for comparison of FPM concentrations between the sexes. All male sample concentrations were similar to the FPM concentration range established for nonpregnant females.

Thirty-five females had at least two fecal samples collected during the sampling period, with 28 having at least two samples collected on different days (see Supplementary Material Tables S1 and S2.). We found that for 25 females with the Progesterone EIA kit and 29 females with the Progesterone Metabolite kit, all individual samples were categorized with the same pregnancy status. Ten females with the Progesterone EIA kit and six females with the Progesterone Metabolite kit had at least one sample with a different pregnancy status categorization as compared to all other individual samples. In the linear mixed models for the Progesterone EIA kit and Progesterone Metabolite kit, the coefficient estimate for date was 0.02, indicating that FPM concentrations of females, both those categorized as nonpregnant and those categorized as preg-

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TABLE 1. Pregnancy status categorization of female elk (n=62) at the North Cumberland Wildlife Management Area in Tennessee, USA, and male elk (n=7) fecal samples collected on the landscape in 2019 using Progesterone EIA and Progesterone Metabolite kits (Arbor Assays). Pregnancy categorization was based on 95% confidence intervals (CI) of fecal progesterone metabolite concentrations from determined nonpregnant and pregnant captured female elk (n=16) in 2019 and 2020. Females with fecal progesterone metabolite concentrations between the ranges of nonpregnant and pregnant were categorized as undetermined.

Kit	Nonpregnant		Undetermined		Pregnant		Males	
	% of Females (n)	Range (ng/g)	% Females (n)	Range (ng/g)	% Females (n)	Range (ng/g)	% Females (n)	Range (ng/g)
Progesterone EIA	40.3 (25)	(81.45–294.24)	6.5 (4)	(354.99–384.35)	53.2 (33)	(416.18–9140.84)	N/A[a] (7)	(68.26–229.65)
Progesterone Metabolite	35.5 (22)	(56.68–282.43)	1.6 (1)	(331.57–372.68)	62.9 (39)	(379.86–4591.33)	N/A (7)	(105.83–297.88)

[a] N/A = not applicable.

nant, increased as the sampling period progressed. Results from the ANOVA indicated a statistically significant, positive relationship between date and pregnancy status (Progesterone EIA kit: P=0.00001; Progesterone Metabolite kit: P=0.000001). With the Fisher exact test, no difference in pregnancy categorization of samples was detected between the two kits (P=0.34).

DISCUSSION

We found pregnancy status of captured elk in 2019 and 2020 to be similar to the status of elk from Elk Island National Park used for reintroduction to Tennessee (Muller et al. 2004). Pregnancy rates in elk vary widely among populations. In eastern elk populations, pregnancy rates were reported between 85–90% in Kentucky, USA (Kentucky Department of Fish and Wildlife Resources [KDFWR] 2019) and 72–73% for elk ≥3 years old in the Great Smoky Mountains National Park, USA (Murrow 2007). Western US elk populations pregnancy rates have been reported between 50–85% (Trainer 1971; Stoops et al. 1999; Creel et al. 2009; Proffitt et al. 2014).

Physical condition, nutrition, age of females and males, environment, and population density all affect reproduction and rate of pregnancy (Cook et al. 2013; Morano et al. 2013; Proffitt et al. 2014). None of the captured yearling elk at the NCWMA were pregnant, but yearling pregnancy is highly variable among different elk populations. In Kentucky, pregnancy rates in yearling elk were 10% in 2016, increasing to 71% in 2019 (KDFWR 2019). Pregnancy rates below 80% in adult elk 3.5–7.5 yr of age may indicate reproductive issues within the population, as adult females are expected to be highly fertile (Raedeke et al. 2002). Noyes et al. (1997) found higher pregnancy rates in elk populations with mature bulls as the predominant breeders. Male age structure and pregnancy status across age classes should be evaluated at the NCWMA as potential influencers of reproductive potential.

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Use of captured females of known age and pregnancy status (as determined from PSPB testing) enabled us to establish a reference range for categorizing females with an unknown pregnancy status from fecal samples collected noninvasively across the landscape. Genetic analysis enabled differentiation of noninvasively collected feces between individuals and for sex, similarly to successful applications in reindeer (*Rangifer tarandus*; Morden et al. 2011). We were unable to differentiate between age classes; therefore, we may have inadvertently included female calves and yearlings in our analysis, and this may have inflated the number of females categorized as nonpregnant. Differentiating adult female age classes would improve pregnancy assessment. Using fecal pellet morphometrics for determining age class has been evaluated in reindeer (Morden et al. 2011) and captive mule deer (*Odocoileus hemionus*; Sánchez-Rojas et al. 2004) and may provide a cost-effective alternative to observational methods, especially in heavily forested or mountainous areas. Further research evaluating the efficacy of using fecal pellet morphometrics to differentiate age classes in elk is warranted. Our methodology, however, allowed us to successfully provide a minimum number of pregnant females (53.2% with the Progesterone EIA kit and 62.9% with the Progesterone Metabolite kit) in the population with the use of noninvasive fecal sampling.

White et al. (1995) reported that the most accurate measure for determining pregnancy status in elk was through free progesterone and a progesterone metabolite, pregnanediol-3-glucuronide, in multiple fecal samples collected during late gestation. We collected samples during the mid- to late gestational period of the NCWMA elk and saw significantly higher FPM concentrations in females categorized as pregnant versus nonpregnant. From females with repeat samples across the collection period, we identified an increase in FPM concentrations as sampling continued into late gestation.

While a single sample may be used to determine pregnancy status during the late gestational period of elk, it does not reflect any physiologic changes that may occur after sample collection. Monitoring individual FPM concentrations annually or across the gestational period on an individual basis may improve population level assessment by monitoring fluctuations or changes in individual pregnancy status. A majority of females with multiple fecal samples collected during our study had no change in pregnancy status and relatively similar FPM concentrations across individual samples. We were able to consistently categorize pregnancy status for 71% of females with the Progesterone EIA kit and 83% of females with the Progesterone Metabolite kit using at least two samples collected from an individual.

Ten females with the Progesterone EIA kit and six females with the Progesterone Metabolite kit had at least one inconsistent sample with a different pregnancy status categorization as compared to all other collected samples. Issues during fecal extraction or pipetting might explain the dissimilarities in these samples. The Progesterone Metabolite kit had higher %CV values as compared to the Progesterone EIA kit for females, with inconsistencies across both kits and sample FPM concentrations, and therefore higher variability in pregnancy status categorization. It is possible that the Progesterone Metabolite kit may be more sensitive to human error occurring in the assay, possibly leading to higher %CV values in duplicates. Some samples were extracted twice between kit analyses due to unusable %CV values or percentage of binding during initial assays, or time between kit analyses was >30 days. Therefore, errors during fecal extractions or assaying, as well as using different extractions for each kit, potentially contributed to the dissimilarities in samples for females with inconsistent FPM concentrations or categorization by kit. Ensuring accuracy in sample processing and analysis and reducing %CV values in duplicates will help improve consistency of FPM concentration analyses and pregnancy status categorization of females with multiple samples collected across gestation (Palme et al. 2013).

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Assay validation tests for both EIA kits indicated reliable measurements of hormones present in the fecal extracts without being influenced by extraneous components within the extract (Morden et al. 2011). According to manufacturer data for cross-reactivity with progesterone and similar closely associated steroids, the cross-reactivity with progesterone for both kits has been shown to be 100%. Cross reactivity for 3β-hydroxy-progesterone with the Progesterone EIA kit has been shown to be 172%, 188% for 3α-hydroxy-progesterone, 147% for 11α-hydroxy-progesterone, and <7% for pregnenolone, corticosterone, and androstenedione (Arbor Assays). Cross-reactivity for any closely associated steroids using the Progesterone Metabolite kit has been shown not to exceed 62% (Arbor Assays).

In our study, comparison of the two EIA kits indicated no significant difference in FPM concentrations between results of the Progesterone EIA kit and the Progesterone Metabolite kit. The Progesterone EIA kit showed higher precision compared to the Progesterone Metabolite kit. However, more females were categorized as undetermined as compared to the Progesterone Metabolite kit. Similarities between the kits resulted in no statistically significant difference between pregnancy status categorization and both are seemingly valid for measuring FPM concentrations in elk feces to determine pregnancy status. However, the Progesterone EIA kit had lower intra-assay and interassay %CV between replicates, indicating a potentially better option for FPM evaluation.

Determining pregnancy status of elk at the NCWMA provides updated information on reproductive parameters that have not been evaluated since initial reintroduction. More research to investigate methods for determining age class of female fecal samples is warranted. Continued monitoring of pregnancy in conjunction with subsequent research on recruitment may provide valuable information needed to assess population growth potential.

ACKNOWLEDGMENTS

This research was funded by the Tennessee Wildlife Resources Agency and supported by the United States Department of Agriculture National Institute of Food and Agriculture McIntire Stennis project (TEN00MS-113) and the Department of Forestry, Wildlife and Fisheries at the University of Tennessee. We thank personnel at the NCWMA, our technicians C. Stelly, N. Pershyn, and A. Dwornik, and personnel at Arbor Assays for their time and support.

SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-22-00041.

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Submitted for publication 31 March 2022.
Accepted 8 August 2022.

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