DIAGNOSIS OF PREGNANCY AND TWINNING IN MOOSE BY ULTRASONOGRAPHY AND SERUM ASSAY

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ABSTRACT: We evaluated transrectal ultrasonography and serum assay for detecting pregnancy in captive and wild moose (*Alces alces*). Ultrasonographic determination of twinning appeared most feasible during days 30-80 of gestation (4 November - 24 December). During December, January, and March, pregnancy, but not twinning, was reliably detected ultrasonographically; diagnosis was confirmed by the presence of a fetus or placentomes. In addition, serum was assayed for pregnancy-specific protein B (PSPB). During December, January, and March, both techniques were 100% accurate in diagnosing pregnancy. However, accuracy of diagnosis during November was 95% and 90% by ultrasound and PSPB assay, respectively, based on our assumption that false positives did not occur with ultrasonography. Detection of the presence of a conceptus in utero eliminates calf detection biases associated with post-partum assessment of moose population productivity.

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Population productivity is directly linked to the nutritional carrying capacity of habitat. In moose, the pregnancy rate of yearlings and the twinning rate of adult cows are potential indicators of habitat quality (Franzmann and Schwartz 1985). However, accurate assessment of pregnancy rates in wild moose can be problematic when based solely on post-partum field observations. Moose often calve in dense cover and neonatal losses from predation (Stephenson and Van Ballenberghe 1995; Ballard *et al.* 1981) and other causes may occur prior to detection of calves. In addition, low bull:cow ratios in hunted populations may prevent fertilization of some cows (Schwartz *et al.* 1994).

Approaches for estimating pregnancy rates in moose populations have involved sampling both live animals and carcasses, as well as analyzing fecal steroids. Schwartz and Hundertmark (1993) conducted fetal counts of cows killed by automobiles. Haigh *et al.* (1982) used rectal palpation to diagnose pregnancy in moose during March. In addition, serum assays have determined pregnancy status based on the levels of progesterone (Haigh *et al.* 1982) and Pregnancy-Specific Protein B (PSPB; Haigh *et al.* 1993). Monfort *et al.* (1993) correctly identified pregnancy status in 85% of captive moose by monitoring fecal progesterone.

Although PSPB has been identified in moose (Haigh *et al.* 1993), we wished to further assess the accuracy of the technique using captive animals of known pregnancy status and by examining animals earlier in gestation. An additional objective was to evaluate real-time ultrasonography for diagnosis of pregnancy and twinning status.

METHODS

Captive moose (n = 21) used during January, March, and Novemer 1994, and January 1995 were maintained at the Kenai Moose

Research Center, on the Kenai Peninsula, Alaska (60°N, 150°W). In addition, wild moose (n = 21) were captured during November and December 1994 in the Nelchina Basin, Alaska (62°N, 147°W) with either a net gun or Palmer Cap-Chur darts fired from a helicopter (Hughes 500). Except for animals that were net-gunned and manually restrained, cows were immobilized with a carfentanil citrate/xylazine hydrochloride mixture and tranquilization was reversed with naltrexone hydrochloride (Schmitt and Dalton 1987). Captive animals determined to be nonpregnant were euthanized and necropsied to verify pregnancy status. The status of captive animals determined to be pregnant was confirmed at the time of parturition. Actual pregnancy status of wild cows was determined using field observations at the time of parturition, as well as agreement with ultrasound and PSPB results.

Serum was obtained from blood collected by jugular venipuncture and was stored frozen (-20 C). PSPB presence in the serum was determined by a double antibody radioimmunoassay (Sasser *et al.* 1986) as applied to moose (Haigh *et al.* 1993). PSPB, isolated from ruminant placentas (Butler *et al.* 1982, Willard *et al.* 1995), is produced by the binucleate cells of the fetal trophectoderm (Eckblad *et al.* 1985). Moose serum was added to assay tubes containing radio-labeled (^{125}I) PSPB isolated from cattle placentae and rabbit antibodies to bovine PSPB. The level of binding of ^{125}I-PSPB was compared to a standard curve developed from bovine PSPB. Binding of radioiodinated bovine PSPB to PSPB antibodies remained near 100% for serum from nonpregnant moose. However, binding of <93% was considered indicative of the presence of moose PSPB and thus pregnancy. Because the moose antigen cross-reacts incompletely with antibodies to bovine PSPB, the moose PSPB concentrations were not measured quantitatively, but a qualitative determination was provided.

Transrectal ultrasonography was used to detect the presence and number of fetuses. Ultrasonic examinations were performed with cows positioned in sternal or lateral recumbency for chemically immobilized and manually restrained animals, respectively. Methyl cellulose, used routinely as a sleeve lubricant for transrectal palpation in large animals, was satisfactory as a lubricant and a coupling agent between the transducer and rectal mucosa in moose. Scanning was conducted with either an Aloka 210 (Corometrics Medical Systems, Inc., Wallingford, Conn.) or an Aloka 500 (Overseas Monitor Corp., Richmond, B.C., Canada) portable ultrasound unit with a 5 MHz linear-array transducer that was powered by a portable, rechargeable 12-volt battery. The ultrasound unit was connected to an 8 mm video cassette recorder to record ultrasound images. The transducer was maneuvered by intrarectal placement of the operator's gloved hand so that the uterine body and both uterine horns were systematically scanned. Definitive diagnosis of pregnancy required identification of either the conceptus or placentomes (Fig. 1), however, a presumptive diagnosis was made if intrauterine fluid and membranes were detected.

Differences between years in twinning rates detected in utero by ultrasonography versus post partum detection of twin calves were tested using chi-square analysis.

RESULTS AND DISCUSSION

Serum from 17 captive cows was assayed for PSPB during 11-25 January and 14 March (Table 1); pregnancy diagnosis was correct 100% of the time. Of these 17 cows, 13 were examined using ultrasonography which also was 100% accurate at pregnancy diagnosis (Table 1). Four additional captive cows were examined using ultrasonography on 12 November; 1 was not pregnant, 1 carried a single fetus, and 2 carried twins. The entire reproductive tract was readily imaged in cows during November and although ovaries were

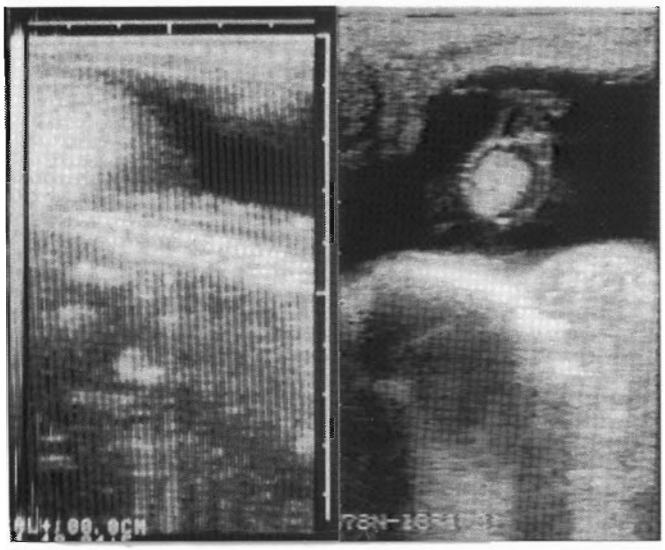

Fig. 1. Ultrasonograms of the moose conceptus during day 40 and day 107 (ages approximate) of pregnancy. Left: The 40 day fetus and fetal membranes appear suspended in amniotic fluid (nonechogenic). Right: The vertebral column (echogenic) of the 107 day fetus is prominent. In addition, a placentome (echogenic) is evident on the upper left side of the image (25 mm in diameter), as well as amniotic fluid (nonechogenic).

scanned in some individuals, we did not consistently scan them. Ultrasonography could not be used to distinguish singleton versus twin pregnancy during January and March because of the larger size of the fetus and extent to which the gravid uterus had descended into the abdomen beyond the signal penetration of the 5 MHz transducer. By day 100 of gestation, the fetus begins to increase in mass rapidly (Schwartz and Hundertmark 1993).

Twenty-one wild cows were examined using ultrasonography and tested for PSPB (Table 2). During 6-7 December, results of the ultrasound and PSPB were in agreement for all 6 animals; however, twinning diagnosis was uncertain due to the distended gravid uterus and less experienced sonographers at this time. During 15-20 November, there were 3 discrepencies between pregnancy di-

Table 1. Percent correct assessement of pregnancy in captive moose as determined by pregnancy-specific protein B (PSPB) in sera and ultrasonography, during 1994-1995 at the Kenai Moose Research Center, Alaska.

Month Pregnancy Status[a]	N	PSPB (% correct)	N	Ultrasound (% correct)
January				
Known Pregnant	10	10 (100)	10	10 (100)
Known Nonpregnant	5	5 (100)	5	5 (100)
March				
Known Pregnant	7	7 (100)	3	3 (100)
Known Nonpregnant	0	0 (100)	0	0 (100)

[a]Determined by either necropsy or successful calving.

Table 2. A comparison of pregnancy diagnosis in wild moose as determined by pregnancy-specific protein B (PSPB) in sera and ultrasonography, during November and December 1994 in the Nelchina Basin, Alaska.

Month Ultrasound	N	PSPB Pregnant	PSPB Nonpregnant
November			
0 Fetuses	4	1[a]	3
1 Fetus	7	5	2[b]
2 Fetuses	4	4	0
December			
Pregnant	5	5	0
Nonpregnant	1	0	1

[a]Interpreted as a false negative by ultrasonography.
[b]Interpreted as false negatives by PSPB assay.

agnoses made with the two methods. We interpreted these as 2 false negatives by the PSPB test (90% accuracy) early in pregnancy, and one false negative by ultrasound examination (95% accuracy). Two of these 3 serum negative cases resulted in a calf born the following spring, while in the third case a distinctive ultrasound image of a calf and a negative PSPB reading were observed, but no calf was found in daily monitoring of the female at the expected time of parturition. Four of 11 moose examined in November (38%) were carrying twin fetuses, which was significantly higher than the twinning rate at parturition the previous spring (9% of 77 parturient moose, $\underline{P} = 0.01$). One of the 3 surviving females with twin fetuses produced a single calf at parturition, but the calf died within 2 days. Although many studies are unable to determine how such losses occur, our sightability was very high during calving season in the open habitats of the Nelchina Basin (Testa, unpubl. data) and females often tended a dead calf for several days. Thus, intrauterine or early neonatal mortality likely explains the discrepancy between ultrasound and calving results in this study.

Ultrasonography, when conducted between approximately day 30 and 80 of gestation (based upon an average conception date of 5 October; Schwartz and Hundertmark 1993), permits diagnosis of pregnancy and twinning in moose. The transducer emits sound waves that respond differently to various tissue types (Ginther 1986). Echogenic tissues, which reflect the signal, appear whitish (e.g., muscle, bone). Nonechogenic materials, such as fluids, appear black. Detection of the fetal heartbeat also aided in diagnosis; in cattle, the fetal heartbeat can be detected by day 22 of gestation (Curran *et al.* 1986). Ultrasonography permitted accurate determination of pregnancy status beyond day 80 but not twinning because of the distended gravid uterus. Beal *et al.* (1992) suggested that in cattle there is little advantage of ultrasonography over rectal palpation for pregnancy diagnosis beyond day 60. However, ultrasound offers tremendous potential in other areas of reproductive biology such as for examination of ovarian anatomy and function (Pierson and Ginther 1988), as well as for documentation of fetal characteristics (Curran *et al.* 1989; Kastelic *et al.* 1988; Griffin and Ginther 1992). Furthermore, Adams *et al.* (1991) noted that with more species-specific experience and greater animal conditioning (in the case of captive nonimmobilized animals), the ability to observe reproductive events by transrectal ultrasonography is greatly

enhanced.

In projects where pregnancy diagnosis is of interest, PSPB assay accurately determines pregnancy status from sera without requiring specialized training or equipment. Because of the potential for misdiagnosing second estrus pregnancies, it may be preferable to delay testing for PSPB until late November. Reliable detection of pregnancy in cattle and sheep on a herd or flock basis using PSPB assay cannot be made until day 28 (Humblot *et al.* 1988) and day 21 (Willard *et al.* 1995) after breeding, respectively. Willard *et al.* (1995) recently developed a quantitative PSPB assay for domestic sheep that permitted detection of fetal twins with up to 82% accuracy. Potentially, PSPB can be isolated from moose cotyledonary tissue to develop antibodies and a standard curve for moose that may permit quantitative assessment of PSPB in moose sera. Consequently, a quantitative assay may enable detection of fetal twins.

Determination of pregnancy status in moose enables evaluation of embryonic, fetal, and neonatal losses; events that have not been previously accessible. Late-term abortions, still-births, and neonatal mortality that occur as a result of poor maternal nutrition (Schwartz, unpubl. data) or stress can result in inaccurate estimation of fertility if pregnancy rates are based solely on post-partum field observations. Additional research needs to be conducted with captive animals of known pregnancy status to fully determine the potential of these procedures.

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