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Efficacy of Pregnancy-Specific Protein B Assay to Detect Pregnancy and Lambing Rates in Sheep

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Summary

Early and accurate identification of pregnancy and lambing rate provides sheep producers many advantages for management decisions that improve flock productivity. The objective of this study was to investigate the efficacy of a commercial pregnancy-specific protein B (PSPB) ELISA assay to predict pregnancy and lambing rate in sheep. On days 20, 25, 30, 40, and 60 postbreeding, blood samples were collected from Columbia and Hampshire ewes. Dorset and Katahdin ewes were sampled 49, 63, and 77 days post ram introduction. Lambing records were used to verify date of conception. Samples were processed using the quantitative BioPRYN®assay for sheep and goat. BioPRYN® classification was 99 percent accurate for pregnant ewes tested after the first month of gestation (i.e., greater than 30 days). For ewes that did not lamb, 90 percent were classified as open and the remaining 10 percent were either misdiagnosed or lost the pregnancy prior to parturition. Ewes carrying multiple pregnancies had greater serum PSPB concentrations than singleton pregnancies from d 40 to d 69 of pregnancy. Effect of breed was detected for serum concentrations of PSPB from d 40 to d 79 of pregnancy. This research indicates that the BioPRYN® test is an effective tool to identify pregnancy in sheep. This test could provide estimates of lambing rates; however, variation in PSPB concentrations due to stage of pregnancy and breed of sheep must be factored into this analysis.

Key words: Pregnancy-Specific Protein B, Sheep, Fetal Age, Pregnancy Rate, Breed

Introduction

Accurate identification of pregnancy status and lambing rate in sheep provides managers several options to increase flock productivity, including reducing management requirements by culling non-pregnant ewes and feeding ewes appropriate diets based on fetal age and number of offspring. By identifying the pregnancy status of ewe lambs (i.e., 9 months of age), producers have the opportunity to market the ewes at a younger age rather than waiting until they are older and classified as mutton (i.e., 12 months of age). Daily feed requirements are 50 percent and 80 percent greater during late gestation compared to maintenance for single and twin-bearing ewes, respectively (NRC, 2007). Additionally, neonatal lamb loss was 85 percent greater in ewes that gave birth to multiple lambs rather than singles (Rowland et al., 1992); therefore, to improve lamb survival, twin-bearing ewes can be provided separate shed-lambing facilities or a separate lambing paddock depending upon the management system.

Ultrasonic imaging is the most common method to determine pregnancy status in the commercial sheep industry, but is often expensive due to the equipment and trained technicians required. In contrast, a blood test allows for flock managers to take samples without expensive equipment or trained technicians. BioTracking, LLC developed a commercially available, pregnancy-specific protein B (PSPB) test for pregnancy in ruminants called BioPRYN® (BioTracking, LLC, Moscow, Idaho), which is completed using an enzyme linked immunosorbent assay (ELISA). The objectives of this research were to determine the efficacy of the BioPRYN® test to predict pregnancy depending on stage of pregnancy and to determine if the test can predict lambing rate in sheep.

Materials and Methods

All experimental protocols were approved by the North Dakota State University (NDSU) Animal Care and Use Committee. Columbia and Hampshire ewes (n = 34 and n = 31, respectively) from the NDSU sheep unit were exposed to intact rams equipped with marking harnesses beginning on August 15, 2011. Two weeks prior to breeding, the Columbia and Hampshire ewes were moved to a drylot and received alfalfa hay (3 kg/ewe) and a 14 percent CP concentrate ration (1 kg/ewe) daily. Breeding marks were identified and recorded. On days 20, 25, 30, 40, and 60 post-breeding, blood samples were collected to determine PSPB concentrations. Ewes that rebred were reassigned new bleeding dates based on the most recent breeding mark. Dorset and Katahdin ewes (n = 68 and n = 24, respectively) were managed on smooth-bromegrass and alfalfa-mixed paddocks and were exposed to intact rams on September 27, 2011. All ewes were single-sire mated to rams of similar breed type to the ewe and at a ewe-to-ram ratio not exceeding 35:1. Blood samples were taken from all ewes d 49, d 63, and d 77 post-ram introduction. Lambing records were taken to determine breeding dates and lambing rates.

Blood samples were collected via jugular venipuncture into 10 mL serum tubes (BD Vacutainer Serum, Becton, Dickinson and Company, Franklin Lakes, N.J.) and immediately placed on ice. Samples were centrifuged at 4°C for 30 min at 1,500 x g, and serum was transferred into plastic 2.0 mL microcentrifuge tubes and frozen at -20 °C until assayed. After all samples were collected, serum was shipped to BioTracking for analysis.

Samples were processed using the quantitative BioPRYN® assay for sheep and goats commercially available through BioLaboratories LLC (Moscow, Idaho). All samples were classified as **open** (less than 15 ng/mL PSPB), **retest** (15 to 30 ng/mL PSPB), or **pregnant** (greater than 30 ng/mL PSPB) based on the standard recommendation from the BioPRYN® test. Concentrations of PSPB for each ewe were determined by least-squares linear regression using known concentrations of purified PSPB as standards.

All samples were classified as either taken from ewes that **did not lamb** (within 150 days post-sample collection) or taken from **pregnant** ewes based on lambing date. Additionally, all samples taken from pregnant ewes were assigned a day of pregnancy number. Day of pregnancy was known for Columbia and Hampshire ewes that lambed within 145 d and 152 d post breeding. However, breeding dates were not collected on Dorset and Katahdin ewes, plus some Hampshire and Columbia ewes lambed to a subsequent breeding event, therefore their breeding date was assigned as 150 d (average gestation of known breeding dates) prior to lambing.

General linear model procedures of SAS (SAS Inst., Inc., Cary, N.C.) were used to analyze serum concentrations of PSPB. To determine the earliest that the BioPRYN® test would detect pregnancy, samples were classified into five subcategories based on BioLaboratories recommendations and prior dates that would be relevant to sheep producers: **1) Did Not Lamb**; samples taken from ewes that did not lamb, **2) < 20 d**; samples taken from 0 days to 19 days post breeding, **3) 20 d - 24 d**; samples taken between from d 20 to d 24 post breeding, **4) 25 d - 29 d**; samples taken from d 25 to d 29 post breeding, and **5) > 30 days**; samples taken from day 30 to day 79 post breeding. These categories were chosen specifically because of prior knowledge of the testing limitation (~30 days), to divide up samples that were taken specifically at 20 d , 25 d, and 30 d, and to include other random samples, for which day of pregnancy was determined based on lambing date. Categorization of testing dates allows producers to make decisions based on days instead of estimations based on a regression equation.

To test the differences in PSPB concentrations among breeds and number of lambs born, samples were divided into ten-day categories through d 80; no samples were taken after this day. This categorization was done to test for differences over time and allow for easy time-point comparisons that are relevant to sheep producer's different management systems. Each block of time was examined independently and actual day of pregnancy was included as a covariate. Least squares means are reported, and differences were considered significant at $P \leq 0.05$.

Results and Discussion

Accuracy by Stage of Pregnancy

The PSPB assay accurately detected 90 percent of ewes that did not lamb (Table 1). We hypothesize that the 10 percent that were classified as recheck or pregnant were pregnant at the time of blood sampling; however, lost the pregnancy prior to parturition. In agreement with our hypothesis, estimates of embryonic or fetal loss have averaged 30 percent in sheep (Bolet, 1986) and 20 percent of embryos have been documented

Table 1. Percentages of samples classified as open, recheck or pregnant by pregnancy-specific protein (PSPB) assay.

	BioPRYN® Classification[1]		
Ewe Pregnancy Status[2]	Open	Recheck	Pregnant
Did Not Lamb	90.00	3.75	6.25
Pregnant			
< 20 d	100.0	0.0	0.0
20 - 24 d	58.3	41.7	0.0
25 - 29 d	0.0	16.7	83.3
> 30 d	0.0	1.4	98.6

[1] BioPRYN® classifications are open (less than 15 ng/mL PSPB), retest (15 to 30 ng/mL PSPB), or pregnant (greater than 30 ng/mL PSPB).
[2] All PSPB samples were included in this table. There were 80, 24, 48, 48, and 365 samples from ewes for each respective row/category (top to bottom).

to be lost after d 25 of pregnancy (Dixon et al., 2007). Although, it was not a part of the original design of the experiment, there were 24 samples taken prior to d 20 of pregnancy, and 100 percent of these samples were classified as open by the BioPRYN® assay. Pregnant ewes were classified as **open** (58 percent) or **recheck** (42 percent) by the assay during the d 20 to d 24 range. From d 25 to d 29 of pregnancy, the assays classified 83 percent and 17 percent of pregnant ewes as pregnant and recheck, respectively. The PSPB assay classified 99 percent of all pregnant ewes as pregnant that were sampled at 30 days or later of pregnancy and the other 1 percent was classified as recheck. This research confirms previous results (Willard et al., 1995) using radioimmunoassay for PSPB and laboratory recommendations that ewes should not be tested prior to 30 days post breeding to confirm pregnancy. Additionally, these results agree with similar PSPB assays conducted in cattle (Romano and Larson, 2010).

Number of Lambs Born

In Table 2, concentrations of PSPB are reported over time, and comparisons are established between ewes that gave birth to single, twin, and triplet births. No differences *(P > 0.09)* were detected for serum PSPB concentration taken from d 0 to d 19 post-estrus among ewes that gave birth to one, two, or three lambs. On d 20 to d 29, serum PSPB concentrations were greater *(P < 0.01)* in ewes that gave birth to twins compared to singletons; whereas, no differences were detected for ewes that gave birth to triplets compared to either single- or twin-bearing ewes (*P = 0.10* and *P = 0.99*, respectively). On d 30 to d 39 and d 70 to d 79, there was a tendency (*P = 0.06* and *P = 0.07*, respectively) for serum PSPB concentrations to be greater for ewes that gave birth to multiple lambs compared to ewes that gave birth to one lamb. From d 40 to d 69, ewes that gave birth to two lambs had on average 25 percent greater *(P < 0.05)* serum PSPB concentrations than ewes that gave birth to one lamb. Regardless of day of pregnancy, no differences *(P > 0.10)* in serum PSPB concentrations were detected between ewes that gave birth to two or three lambs. On day 40 to day 59 and day 70 to day 79, serum PSPB concentrations were greater *(P < 0.05)* for ewes that gave birth to three lambs than ewes that gave birth to one lamb; however, no differences *(P = 0.45)* were detected between d 60 and d 69. In agreement with Willard et al. (1995), these data indicate that single and twin pregnancies can be estimated by PSPB concentrations after d 40 of pregnancy; however, actual age of pregnancy must be known to adjust for changes in PSPB throughout gestation. Our data do not indicate that twin and triplet pregnancies could be predicted. Only 14 ewes gave birth to three lambs (2 Columbia ewes, 5 Dorset ewes, 1 Hampshire ewe, and 6 Katahdin ewes). Too few ewes gave birth to triplets to detect modest differences among multiple pregnancies. However, incomplete pregnancy loss, during or after these samples were collected, is common in sheep (Dixon, 2007), and this could have contributed to added variation in this data set. Wallace et al. (1997) reported that PSPB concentrations increase during rapid placental growth, which implies that PSPB is released by the binucleate cells of the trophectoderm in concentrations proportional to placental mass. Total placental mass is greater in twin versus singleton pregnancies (Alexander, 1974) indicating a rationale for higher PSPB concentrations in twin pregnancies.

On d 0 to d 9, d 20 to d 29, and d 30 to d 39, no differences among breeds *(P > 0.11)* were detected for serum PSPB concentrations (Table 1). On d 10 to d 19, Hampshire ewes had greater *(P < 0.04)* PSPB concentrations than Columbia and Dorset ewes. We are cautious to make any

Table 2. Serum pregnancy-specific protein B (PSPB) concentrations (ng/mL) at different stages of pregnancy in ewes with different numbers of lambs born.

	Number of Lambs Born				
Day of Pregnancy[1]	Single	Twin	Triplet	SEM	*P-value*
0-9	6.2	5.8	-	0.5	0.47
10-19	6.1	3.9	-	1.2	0.05
20-29	23.9[a]	30.7[b]	30.6[ab]	4.3	<0.01
30-39	59.8	68.9	70.9	6.5	0.06
40-49	61.3[a]	77.9[b]	80.8[b]	6.1	<0.01
50-59	55.4[a]	75.8[b]	89.9[b]	9.5	<0.01
60-69	83.6[a]	100.4[b]	100.4[b]	8.4	<0.01
70-79	66.0	85.7	88.4	12.5	0.07

[a,b] Row means with different superscripts differ (*P < 0.05*).
[1] All pregnant ewe PSPB samples were included in this table. There were 11, 12, 97, 77, 105, 51, 98, and 33 samples for each respective row category (top to bottom). If fewer than two samples were taken within a number of lambs born category for a particular day of pregnancy range, means were not presented.

inferences based on this detected difference because the mean values are approximately 1/3 the threshold for pregnancy detection established by BioLaboratories, and only 12 samples were taken during this time period. On d 40 to d 49 and d 60 to d 69, Columbia ewes had greater *(P < 0.03)* concentrations of PSPB than all other breeds. During the same time frame, Dorset ewes had greater *(P < 0.01)* concentration of serum PSPB than Katahdin ewes and Hampshire ewes were not different *(P > 0.09)* from either Dorset or Katahdin. On d 50 to d 59 *(P < 0.02)* and d 70 to d 79 *(P = 0.03)*, Columbia and Dorset ewes had greater concentrations of PSPB in serum than Katahdin ewes. Willard et al. (1995) also reported differences among breeds for PSPB concentrations. Their research indicated that Columbia ewes had greater concentrations of PSPB than Rambouillet and Targhee ewes. It is unknown why differences in PSPB exist among breeds; however, it is likely a result of differences in placental development or production of PSPB per-unit placental mass, as indicated by Wallace et al. (1997). Of the breeds used in this project, Katahdin and Dorset have a much smaller mature size than Columbia and Hampshire ewes. These large differences in BW and variation in blood-volume-per-unit BW could have an impact on PSPB concentrations; however, there does not appear to be a correlation between PSPB concentrations and breed mature size.

Conclusions

This research demonstrates that the BioPRYN® test is an effective tool to detect pregnancy in sheep after the first month of gestation. Tested after 30 days of pregnancy, 99 percent of pregnant ewes were classified as pregnant and only 6.25 percent of ewes that did not lamb were classified as pregnant, which is likely due to fetal loss. Concentrations of PSPB were dependent on stage of pregnancy, breed of sheep, and lambing rate. Differences in PSPB concentrations were detected between singleton and twin-bearing ewes. This test could be used to estimate lambing rate; however, variation in PSPB contributed by breed and age of pregnancy must be quantified. Additionally, if PSPB can serve as a non-invasive measure of placental development, it could improve scientific investigation towards enhancing or alleviating improper placental/fetal development.

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Table 3. Serum pregnancy-specific protein B (PSPB) concentrations in ewes from different breeds at different stages of pregnancy.

	Ewe Breed					
Day of Pregnancy[1]	Columbia	Dorset	Hampshire	Katahdin	SEM	*P-value*
0-9	6.4	-	5.5	-	0.6	0.17
10-19	5.1[b]	-	7.0[a]	-	1.0	<0.01
20-29	30.8	22.3	28.3	32.2	9.7	0.50
30-39	71.9	71.5	62.3	60.5	7.1	0.11
40-49	89.3[a]	75.5[b]	69.1[bc]	59.5[c]	6.9	<0.01
50-59	110.8[a]	95.6[a]	-	63.5[b]	18.8	<0.01
60-69	121.1[a]	98.1[b]	86.5[bc]	73.4[c]	9.2	<0.01
70-79	-	90.8[a]	-	69.3[b]	9.1	0.03

[a,b,c] Row means with different superscripts differ (*P < 0.05*) within a row.

[1] All pregnant ewe PSPB samples were included in this table. There were 11, 12, 97, 77, 105, 51, 98, and 33 samples for each respective row category (top to bottom). If there were fewer than two samples within a breed for a particular day of pregnancy range, means were not presented.

