Efficacy of pregnancy specific protein B assay to predict pregnancy and pregnancy rate in sheep

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Our objective was to evaluate the effectiveness of the pregnancy specific protein B assay to predict early pregnancy and pregnancy rate in sheep. This test accurately detected flock pregnancy status after 30 days of pregnancy. Multiple pregnancies had greater PSPB concentrations in two of the four breeds.

INTRODUCTION

Most North Dakota sheep production scenarios require substantially more resources, such as feed, labor, and facilities during late gestation and lactation. Early identification of pregnancy and pregnancy rate in sheep provides managers several options to increase flock productivity. First, removal of non-pregnant ewes can increase available resources for pregnant ewes. Second, identification of pregnancy status of ewe lambs (9 months of age) allows producer to market ewe lambs as lamb, instead of waiting until after the lambing season, when ewe lambs will be older than 12 months and are more likely to be classified as mutton. Third, identification of pregnancy rate in sheep allows producer to target feed resources to ewes baring multiple lambs. Twin baring ewes require 30% more feed than singleton baring ewes. Finally, identification of early and late lambing ewes allows producer to target feed resources to ewes during period of greatest need. Late gestation singleton and twin baring ewes require 50 and 80% more feed, respectively, than ewes in early gestation.

Ultrasonic imaging is the most common method to determine pregnancy and pregnancy rate. This technique requires expensive equipment and a highly trained technician. Many states require this technique be conducted by a licensed veterinarian. Qualified technicians or veterinarians are not available to many sheep producers or costs associated with travel to remote locations are prohibitive. BioTracking, LLC developed a commercially available pregnancy-specific protein B (PSPB) test for pregnancy in cattle. The test was named BioPRYN®, with PRYN standing for "Pregnant Ruminant Yes No". This technology was licensed by BioTracking from the University of Idaho and the assay was converted to an enzyme-linked immunosorbent assay (ELISA). The PSPB test was later developed for sheep and goats.

Our objectives for this study were to determine the earliest day of pregnancy that the BioPRYN test could accurately detect pregnancy status and pregnancy rate.

PROCEDURES

All experimental protocols were approved by the North Dakota State University Animal Care and Use Committee. This study consisted of two different experiments both held at the NDSU Sheep Unit in Fargo, ND.

In Exp. 1, Columbia and Hampshire ewes were exposed to intact rams equipped with marking harnesses on August 15th, 2011. Breeding marks were identified and recorded. On days 20, 25, 30, 40, and 60 post-breeding, blood samples were collected to determine PSPB concentrations. In Exp. 2, Dorset and Katahdin ewes were exposed to intact rams on September 27, 2011. Blood samples were taken from all ewes 7, 9, and 11 weeks post ram introduction. Lambing records were used to verify conception dates and lambing rates.

All blood samples were collected via jugular venipuncture into 10 mL serum tubes (BD Vacutainer Serum, Becton, Dickinson and Company, Franklin Lakes, NJ) 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, samples were shipped to BioTracking for analysis.

Only, 2 Columbia ewes and 1 Hampshire ewe gave birth to triplets. Two Katahdin ewes gave birth to singleton lambs. All five ewes were removed from the analysis because of limited number of ewes within lambing groups.

Pregnancy classification as determined by BioPRYN at different stages of pregnancy was analyzed using the PROC FREQ and CHI-SQUARE function of SAS (SAS Inst., Inc., Cary, NC). Repeated measures of the MIXED procedure of SAS were used to analyze serum concentrations of PSPB. Breed, day of pregnancy, and their interaction were significant in the model; therefore, breed and day of pregnancy sorted and analyzed independently. Data are presented as least squares means and treatment differences were considered significant at $P \leq 0.05$.

RESULTS

To determine the earliest that the BioPRYN test could detect pregnancy, samples were classified into three subcategories: less than 25, between 25 and 30, and over 30 days of pregnancy. There was a significant ($P < 0.01$) interaction between day of pregnancy tested and classification of pregnancy via BioPRYN testing. The test accurately detected pregnancy in 2, 83, and 98% of pregnant ewes when tested less than 25, between 25 and 30, or greater than 30 days of pregnancy, respectively (Table 1).

Concentration of PSPB are presented for Columbia ewes that lambed to singleton and twin pregnancies (n = 14 and 7, respectively; Table 2). Number of lambs born did not have an effect on PSPB concentrations on days 20 and 60 post breeding ($P \geq 0.27$) in Columbia ewes. On days 25, 30, and 40 post breeding, twin pregnancies had greater ($P \leq 0.05$) PSPB concentrations than singleton pregnancies.

Concentration of PSPB are presented for Hampshire ewes that lambed to singleton and twin pregnancies (n = 11 and 7, respectively; Table 3). Number of lambs born did not have an effect on PSPB concentrations on days 20, 25, 30, 40, and 60 post breeding ($P \geq 0.06$) in Hampshire ewes.

Concentration of PSPB taken 49, 53, and 67 days post ram introduction to Dorset ewes that lambed to singleton, twin, and triplet pregnancies (n = 21, 37, and 5, respectively; Table 4). Actual days of pregnancy for the blood samples were back calculated from the lambing date. The average actual days of pregnancy were 40, 54, and 68 ± 7.5 for the three respective sampling dates. Number of lambs born did not have an effect on PSPB concentrations taken 49 days post ram introduction ($P = 0.11$) in Dorset ewes. Twin pregnancies had greater ($P \leq 0.01$) PSPB concentrations than singleton pregnancies on days 53 and 67 post ram introduction. Triplet pregnancies were not different ($P \geq 0.13$) from twin or singleton pregnancies on days 53 and 67 post ram introduction.

Concentration of PSPB taken 49, 53, and 67 days post ram introduction to Katahdin ewes that lambed to twin and triplet pregnancies (n = 13 and 6, respectively; Table 5). Actual days of pregnancy for the blood samples were back calculated from the lambing date. The average actual days of pregnancy were 37, 51, and 65 ± 8.1 for the three respective sampling dates. Number of lambs born did not have an effect on PSPB concentrations taken 49 days post ram introduction ($P \geq 0.06$) in Katahdin ewes.

DISCUSSION

Our first objective was to determine when the assay could accurately detect pregnancy in sheep. Although pregnancy was detected as early as day 20 in one ewe, 72% of pregnant ewes were falsely determined to be open when the test was conducted before 25 days of pregnancy. Testing between day 25 and 30 of pregnancy accurately determined 83% of pregnancies; however, the remaining ewes were determined to be open or required additional testing. Testing for PSPB after 30 days of pregnancy accurately identified 98% of pregnant ewes. One ewe tested open at 30 days of pregnancy; however, the next test identified the ewe as pregnant. Although this study was not designed to identify false positives, we did not have any open ewes that were determined to be pregnant by the PSPB test. There were some ewes that tested pregnant on initial tests but later testing identified the ewe to be open. We hypothesize that this was a result of failed pregnancies.

In agreement with previous research (Willard et al., 1995), breed and age of pregnancy were strongly correlated with PSPB concentrations. These contributing factors make it difficult to randomly take a sample from ewes that have been exposed to rams and determine pregnancy rate. Concentrations of PSPB were not correlated to litter size in the Hampshire and Katahdin breeds; whereas, concentration of PSPB and litter size were correlated in the Columbia and Dorset breed. There may be opportunity for sheep producer of this breed to sort into pregnancy groups by PSPB concentration; however, exact date of breeding must be known. Unfortunately, triplet baring ewes from both the Dorset and Katahdin breed did not express greater PSPB concentration than twin baring ewes.

Although, PSPB testing cannot definitely identify pregnancy rate, there may be opportunity for sheep producers to improve efficiency through this test. First, identification of pregnancy beyond 60 days of gestation was 100% accurate; therefore, ewes that failed to become pregnant or lost a pregnancy can be identified and removed from the flock. Second, individual ewe nutrition and management requirements increase as pregnancy progresses and if multiple

pregnancies are present. Similarly, within a breeding group PSPB concentration were higher in ewes that possessed the oldest pregnancies or multiple pregnancies. Therefore, sorting a group of ewe by PSPB concentration would allow for producers to improve efficiency of feed, labor and facility resources.

IMPLICATIONS

The PSPB test was very effective at detecting pregnancies beyond 30 days and it is very likely to be as good as or better than most ultrasound technicians. Although PSPB concentrations were able to differentiate between single and multiple pregnancies, it was not consistent between breeds and age of pregnancy must be known. Skilled ultrasound technicians would likely be more accurate at detection of pregnancy rate than the PSPB test.

LITERATURE CITED

Willard, J. M., D. R. White, C. A. Wesson, J. Stellflug, and R. G. Sasser. 1995. Detection of fetal twins in sheep using a radioimmunoassay for pregnancy-specific protein B. J. Anim. Sci. 73: 960-966.

Table 1. Number and percent of pregnant ewes classified as open, recheck or pregnant by PSPB test at different days of pregnancy in Exp. 1 and 2

	BioPRYN Classification[1]			
Days of Pregnancy	Open	Recheck	Pregnant	*P*-Value
< 25 days	31(72)	11(26)	1(2)	<0.01
25 – 30 days	2(3)	11(14)	67(83)	<0.01
≥ 30 days	1(0.3)	7(2)	299(98)	<0.01

[1]BioPRYN classification are open (less than 15 ng/mL), retest (15 to 30 ng/mL), or pregnant (greater than 30 ng/mL).

Table 2. Serum pregnancy specific protein B (PSPB) concentrations in pregnant Columbia ewes[1]

	Number of Lambs Born				
Day of Pregnancy	Singleton	Twin	Triplet	SE	*P*-Value
20	11.6	14.5	.	1.9	0.24
25	33.1[a]	46.2[b]	.	5.0	0.05
30	59.7[a]	80.3[b]	.	6.1	<0.01
40	71.8[a]	90.8[b]	.	4.9	<0.01
60	114.2	125.5	.	10.5	0.13

[1] n = 14 and 7 for singleton and twin pregnancies, respectively.

Table 3. Serum pregnancy specific protein B (PSPB) concentrations in pregnant Hampshire ewes

	Number of Lambs Born[1]				
Day of Pregnancy	Singleton	Twin	Triplet	SE	*P*-Value
20	14.1	12.7	.	3.4	0.76

25	34.5	36.1	.	5.1	0.82
30	56.3	52.7	.	8.2	0.74
40	55.9	69.5	.	5.6	0.06
60	72.9	86.2	.	11.8	0.38

[1] n = 11 and 7 for singleton and twin pregnancies, respectively.

Table 4. Serum pregnancy specific protein B (PSPB) concentrations in pregnant Dorset ewes

	Number of Lambs Born[1]				
Days after Ram Introduction[2]	Singleton	Twin	Triplet	SE	*P*-Value
49	64.4[a]	79.2[b]	69.9[ab]	11.3	0.11
53	77.4[a]	97.5[b]	93.2[ab]	9.3	<0.01
67	79.7[a]	98.4[b]	89.1[ab]	9.4	<0.02

[1] n = 21, 37, and 5 for singleton, twin, and triplet pregnancies, respectively.
[2]The average days of pregnancy were 40, 54, and 68 ± 8.1 for the three respective sampling dates.

Table 5. Serum pregnancy specific protein B (PSPB) concentrations in pregnant Katahdin ewes

	Number of Lambs Born[1]				
Days after Ram Introduction[2]	Singleton	Twin	Triplet	SE	*P*-Value
49	.	66.4	74	6.3	0.35
53	.	67.5	84	9.3	0.17
67	.	65.7	77.7	7.8	0.22

[1] n = 13 and 6 for twin triplet pregnancies, respectively.
[2]The average days of pregnancy were 37, 51, and 65 ± 8.1 for the three respective sampling dates.