PREGNANCY SPECIFIC PROTEIN B AS A PREDICTOR OF GESTATION LENGTH IN ANGUS COWS AND HEIFERS

A Thesis

Presented to the

Faculty of the College of Graduate Studies and Research

Angelo State University

In Partial Fulfillment of the

Requirements for the Degree

MASTER OF SCIENCE

by

CANDIDA KATALINA CHAIREZ

August 2019

Major: Animal Science

PREGNANCY SPECIFIC PROTEIN B AS A PREDICTOR OF GESTATION LENGTH IN ANGUS COWS AND HEIFERS

by

CANDIDA KATALINA CHAIREZ

APPROVED:

Dr. Micheal W. Salisbury

Dr. Chase A. Runyan

Dr. Loree A. Branham

Dr. Jeffrey D. Dailey

August 2019

APPROVED:

Dr. Micheal W. Salisbury
Dean, College of Graduate Studies and Research

ACKNOWLEDGEMENTS

Thank you to all the staff of the Angelo State University ranch who helped collecting blood samples and taking good care of the animals during this study. A special thanks to Dr. Micheal Salisbury, Dr. Chase Runyan, and Dr. Loree Branham for guiding me every step of the way, not only while working on my thesis, but for the last couple of years in the classroom, and for all the essential knowledge I gained throughout that time. I also want to thank my family and friends for all the love and support, especially during challenging times.

ABSTRACT

The objectives of this study were to characterize a relationship between Pregnancy Specific Protein B (PSPB) concentrations and the stage of gestation for cows and heifers and to determine if these concentrations can predict gestation length. Fifty-three multiparous cows and 21 single parity cows were used. The animals were estrus synchronized, bred via artificial insemination, and exposed to a bull to increase conception rates. Blood samples were collected several months throughout gestation and sent to the laboratory to be analyzed through an ELISA. Concentrations of PSPB were recorded for each animal by month and parturition dates. The concentrations measured by optical densities for the month of May had a strong correlation with birth date and birth order ($P < 0.001$). The strength of the correlation began to decrease with time. Calving probability within the first 31 days of calving season was high for May when optical densities were higher than 0.400. The optical densities of the subsequent months show lower possibilities for predicting calving time.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Early pregnancy diagnosis is a critical part in most dairy and beef cattle operation's reproductive management strategies. Reproductive efficiency is one of the primary goals of these operations to maintain and improve productivity. Producers prefer diagnosing pregnancy as soon as possible after breeding in order to decide the future of non-pregnant cows and non-pregnant first-calf heifers. If females have not conceived, the producer may try to rebreed the animal through artificial insemination or by exposing the cow to another bull, if the breeding time will meet the optimal calving interval. In most cases, non-pregnant cows will be culled and removed from the operation. Recently, more cattle operations have begun implementing the use of blood tests to diagnose pregnancy.

One of the companies that offers the ruminant pregnancy tool is BioPRYN® (Moscow, ID). The pregnancy test works by processing blood samples and detecting the concentration of Pregnancy Specific Protein B (PSPB), which is a protein released during gestation. The blood test can be performed as soon as 28 days after breeding. However, there is a postpartum window that cows must meet for the test to be accurate. Residual protein levels from the previous pregnancy can be detected if the cow is less than 73 days postpartum. Several studies have tested the validity and effectiveness of the PSPB test and have concluded it to be reliable and practical as a pregnancy diagnostic tool (Sasser et al., 1986; Love et al., 2017). In addition, studies have made suggestions about the ability of PSPB to predict gestation length in ruminants. Knowledge of gestation stage in a cow or heifer can be a useful tool. Producers can observe the animals around their projected due

dates so that intervention can be provided when needed in case of birthing complications. Furthermore, sorting cows into different groups to optimize their nutritional requirements can be accomplished by knowledge of the gestation length.

OBJECTIVES

The objectives of the study are to:

1) Characterize a relationship between Pregnancy Specific Protein B (PSPB) concentrations and stage of gestation in Angus cows and heifers.

2) Determine if concentrations of PSPB can predict gestation length.

LITERATURE REVIEW

Validity of Pregnancy Specific Protein B as a Ruminant Pregnancy Detection Tool

There are several methods of diagnosing pregnancy in cows. The two most common approaches involve rectal palpation and ultrasound. The more modern method of diagnosis is through the use of blood tests. Blood tests determine pregnancy by testing for baseline concentrations of specific proteins. The protein which has been analyzed and has shown accuracy and reliability in pregnancy diagnosis is the bovine Pregnancy Specific Protein B (PSPB) (Love et al., 2017). Pregnancy Specific Protein B is a pregnancy associated glycoprotein. It is produced by the trophoblastic cells of the placenta, and it is released into the maternal circulation through the bloodstream and plays a role in maintaining pregnancy in bovines (Romano and Larson, 2010; Senger, 1997).

BioPRYN® (Moscow, ID) is one of several companies that provides the pregnancy detection tool for ruminants. Blood samples are processed by an enzyme-linked immunosorbent assay (ELISA) to assess the blood serum's PSPB levels. Producers can collect whole blood from the animals' jugular vein or coccygeal vein via vacuum tubes. Blood samples are then stored in an ice rack and refrigerated until they are shipped to the laboratory.

More cattle operations are integrating the use of blood tests rather than manual pregnancy determination. The advantage of testing for this particular blood protein is obtaining pregnancy diagnosis earlier than through rectal palpation. Rectal palpation can be performed accurately on day 35 post-breeding, and ultrasound can be used on day 28 (Scully et al., 2014). Both methods are accurate and reliable when performed by a skilled technician, yet they lack the cost effectiveness of the PSPB blood test. Testing for the levels of

Pregnancy Specific Protein B provide results as early as 28 days post-breeding, however, cows must be 73 days postpartum because of the potential risk of residual concentrations of PSPB from the previous gestation period (Ergene et al., 2018). In addition, it is recommended that levels be tested 60-70 days post-breeding to diagnose early embryonic death loss.

Pregnancy Specific Protein B and the Potential for Predicting Gestation Length

The use of PSPB as an effective tool for early pregnancy diagnosis has been established. Several studies have mentioned the potential use of Pregnancy Specific Protein B as a predictor of gestation length, and only a few have tested that ability. One of the studies utilized five cows and found that four of the five cows had similar concentrations of PSPB throughout gestation (Sasser et al., 1986). It also mentioned in the discussion that stage of pregnancy may be predicted, based on the PSPB levels. This study also suggested that levels below 5 ng/ml indicate pregnancy length of less than 80 days and values greater than 10 ng/ml would suggest a length greater than 80 days. The implementation of the study provides some guidance in this area of research. However, since the sample size used was small, it leaves more room for variability and can affect the generalizability of the results. Pregnancy Specific Protein B concentrations in other species have also been observed. Ewes were used to find a correlation between PSPB profiles and pregnancy outcome (Wallace et al., 1997). The study was able to establish a correlation between PSPB concentrations and fetal mass at term. As protein levels increased, particularly during the time of pre-partum leading up to parturition, fetal mass also increased. With this association, PSPB levels could reflect fetal mass as an intermediate indicator toward the prediction of gestation stage.

Patel et al. (1998) utilized Holstein cows to observe the levels of Pregnancy Specific Protein B from the first day of standing estrus to one day postpartum. Results showed that levels increased progressively from day 20 post-estrus to 20 days before parturition, and between day 20 to day 10 pre-partum, mean concentrations of PSPB increased significantly. With the dramatic concentration increase around the last three weeks of gestation, parturition time could potentially be approximated. Patel et al. (1995) also mentioned abnormal PSPB concentrations and fluctuations were observed when cows gave birth prematurely to stillborn fetuses. Therefore, fetal viability is a factor that could potentially be predicted by the concentrations as well.

Pregnancy Associated Glycoproteins and Embryonic Mortality

The relationship between pregnancy associated glycoproteins levels and late embryonic mortality has been found to be significantly correlated (Pohler et al., 2016). These proteins were positively associated with the survivability of the embryo. In the study, cows 31 days into gestation that had concentrations below 1.4 and 1.8 ng/mL resulted in 95% chance of embryonic death by day 60. Circulating protein levels seem to be a useful biomarker to predict the likelihood of pregnancy maintenance.

The bovine Pregnancy Specific Protein B blood test as an effective tool for pregnancy diagnosis and is a topic that has been thoroughly studied and established. Further inquiry of the PSPB to predict gestation length is still an area of interest that has not been established. Research studies have collected data showing correlations between PSPB, fetal mass and pregnancy outcome and the progressive increase of concentrations throughout gestation. However, these studies have not evaluated the relationship between the PSPB concentrations and stages of gestation specifically. Most of these studies have been conducted on dairy cows

and other species of livestock such as sheep. Beef cows have had minimal presence in these studies. The integration of beef cows to observe the effect of PSPB on gestation length imposes an interesting focus that could be helpful to producers and potentially provide them an advanced tool that can predict how the pregnancy is proceeding, the stage of gestation, and if there are any complications occurring.

MATERIALS AND METHODS

The study was conducted at the Angelo State University Management, Instruction, and Research Center. The methodology was approved by the Institutional Animal Care and Use Committee Protocol #19-102. Angus cows and heifers (n=74) (53 mature cows have more than one parity and 21 single parity cows) were used in the study. The animals were estrus synchronized with a 7-day CoSync+CIDR®, which is a T-shaped vaginal insert containing progesterone that is safe for use in cattle (Mapletoft, 2002). The cows also received an injection of Gonadotropin Releasing Hormone (GnRH) at this time. The inserts were removed after 7 days and an injection of Prostaglandin (PGF2α) was given. After two days without the insert, cows were bred via artificial insemination (AI) by a trained technician, received a second injection of GnRH and then exposed to a bull after AI. There were three breeding dates. The first breeding date was for all first-calf heifers (day 101 of the year), the second breeding date was for the first group of mature cows (day 103), and the third for the second group of mature cows (day 129). The bull was removed from the cowherd 37 days (day 166) after the last AI breeding date. Blood was collected 13 days after the last breeding day (day 142) via caudal vein and placed in Vacutainer® (Franklin Lakes, NJ) tubes with no anticoagulants or additives. The blood tubes were stored in a refrigerator at 4°C while shipping was organized. The blood samples were shipped to a certified BioPRYN® laboratory to be analyzed through an enzyme-linked immunosorbent assay (ELISA) for an initial pregnancy diagnosis. Blood was collected monthly during gestation after the initial pregnancy diagnosis and concentrations of Pregnancy Specific Protein B (in optical densities) were recorded. Gestation stage was verified with actual parturition dates. Data was analyzed using SAS 9.4 (SAS inst. Inc., Cary, NC) to determine correlation

between predicted gestation day (as determined by blood sample) and actual gestation day as calculated by using date of parturition and counting back to blood sample day. Proc Logistic procedures were also used to evaluate PSPB levels across days with 95% confidence intervals being included in the analysis. The procedures conducted for this study are shown in Table 1.

Table 1. Corresponding Julian Dates of 2018 for Estrus Synchronization, Artificial Insemination, and Blood Collections

Procedure	Julian Day (2018)
Estrus synchronization	91, 93, 119
AI[1] of heifers	101
AI of first group of mature cows	103
AI of second group of mature cows	129
May blood collection	142
June blood collection and removal of bull from cow herd	166
August blood collection	234
September blood collection	270

[1] AI = Artificial Insemination

RESULTS AND DISCUSSION

The mean optical densities (OD), reflecting the concentrations of PSPB, increased from the month of May to June, and from June to August. In September, the mean OD demonstrated a decrease in PSPB concentrations. This data is presented in Table 2.

Table 2. Means, Standard Deviations, Minimum and Maximum Values of Breed Dates (AI or Natural Service), Optical Densities Per Month of Pregnancy Specific Protein B, Birth Date and Birth Order of Angus Cows and Heifers

Variable	Mean	St Dev	Minimum	Maximum
AI[1] date	76.04	15.98	101.00	129.00
Breed day	105.94	30.41	101.00	166.00
OD[2] May	0.34	0.29	0.07	0.81
OD June	0.66	0.33	0.05	1.02
OD Aug	1.06	0.14	0.68	1.25
OD Sept	0.83	0.09	0.69	1.19
Birth day	35.47	18.48	10.00	71.00
Birth order	25.37	14.82	1.00	51.00

[1] AI = Artificial Insemination
[2] OD = Optical Densities

Concentrations of PSPB during gestation in this study increases progressively during the first four months. This is in agreement with Sasser et al. (1986), where the cows observed had PSPB concentrations that increased until after parturition. However, in this study, the concentrations then decrease slightly from August to September with mean optical densities of 1.06 in August reducing to 0.83 in September. Thus, the mean concentrations of PSPB for each month of gestation may not accurately predict the stage of gestation because of the lack of steady increase of PSPB in September. The fluctuation in optical densities between August and September pose the possibility that was addressed by Pohler, about embryonic mortality occurring by day 60 or more of gestation (Pohler et al, 2016). Given that the bull was removed from the cow herd in mid-June, the 60 days after would make August the month

where the majority of embryo losses could occur, subsequently reducing the mean optical densities of PSPB in September.

The relationship between PSPB concentrations and birth dates and birth order using the CORR procedure and Pearson correlation coefficients, was significant for the first month of blood collection. For the month of May, the optical density of PSPB and birth order showed a strong, negative correlation with a coefficient of -0.793 ($P < 0.001$). Birth date and OD of May had a correlation coefficient of -0.780. The strong, negative correlation means that the higher the OD in May, increases the likelihood of the cow birthing first. The correlation coefficient between optical densities for each month and birth date and birth order decreased across time. This data is shown in Table 3.

The mean optical density for the month of May exhibits a strong relationship with birth date and birth order. The concentrations of PSPB for this month provide useful information to the producer. A high OD of PSPB in the first month of gestation can indicate which cows and heifers will calve first. This allows operations to group animals accordingly, adjust nutritional intake leading up to parturition, and keep a close watch on those cows and heifers that are predicted to calve first in the incidence of birthing complications.

Table 3. Pearson Correlation Coefficients and P-values for Breed Dates, Optical Densities Per Month of Pregnancy Specific Protein B, Birth Date and Birth Order for Angus Cows and Heifers (n=74)

Variables	AI Date	Breed day	OD May	OD June	OD Aug	OD Sept	Birth date	Birth Order
AI[1] Date	1.000							
Breed Day	0.328	1.000						
	0.022							
OD[2] May	0.190	-0.915	1.000					
	0.217	<0.001						
OD June	-0.009	-0.295	0.225	1.000				
	0.960	0.095	0.233					
OD Aug	0.040	-0.168	0.160	0.622	1.000			
	0.785	0.253	0.307	0.001				
OD Sep	0.057	0.140	-0.186	-0.130	-0.112	1.000		
	0.704	0.348	0.239	0.479	0.458			
Birth date	0.318	0.849	-0.780	-0.563	-0.404	0.136	1.000	
	0.026	<0.001	<0.001	0.006	0.004	0.363		
Birth Order	0.339	0.855	-0.792	-0.501	-0.364	0.159	0.979	1.000
	0.017	<0.001	<0.001	0.003	0.011	0.287	<0.001	

[1] AI = Artificial Insemination

[2] OD = Optical Densities of PSPB

Probability of Predicting Calving in the First 31 Days of Calving Season

The Proc Logistic statistical procedure was used to determine the probability that the OD for each month will predict a cow or heifer to calve within the first 31 days of the calving season. The probability for the month of May's OD to predict calving increases as the OD increases. This is illustrated in Figure 1. The figure depicts probability of calving with 95% confidence intervals. There is a broad range of probability shown in the figure. The low OD may correspond to non-pregnant cows or those that are recently bred and initially acquiring concentrations of PSPB. An optical density of 0.400 or higher indicates a probability of 0.75 or higher for the month of May, suggesting the likelihood of parturition in the first 31 days.

Figure 2 and Figure 3 for the months of June and August have positive slopes, but the probability of predicting calving time decreases as the months go on. The broad variance in probability persist throughout these months as well. The ODs of September and the probability of calving exhibits a negative trend in Figure 4. This variance is large for ODs of 0.85 to 1.2 which are depicted in the figure. With the variance so large, it will hinder the ability to predict parturition. In addition, given that concentrations of PSPB were not measured after the month of September, it cannot be inferred that the concentration will continue to decrease.

Figure 1. Probability of May Optical Densities of Pregnancy Specific Protein B to Predict Calving in the First 31 Days of the Calving Season

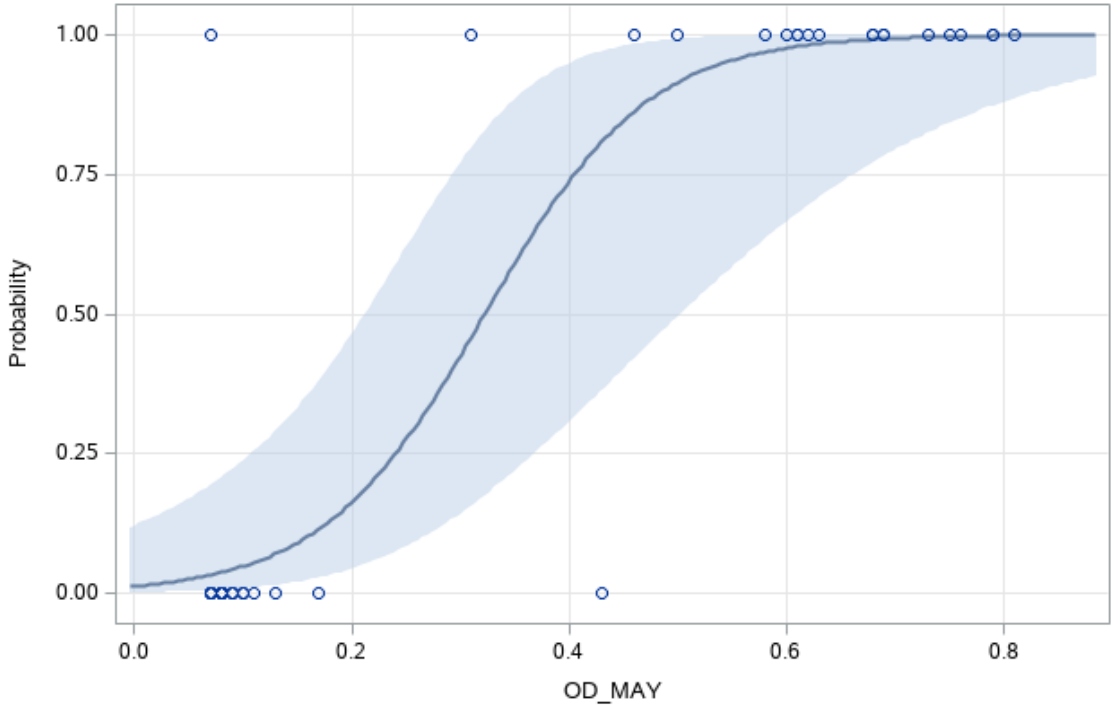


Figure 2. Probability of June Optical Densities of Pregnancy Specific Protein B to Predict Calving in the First 31 Days of the Calving Season

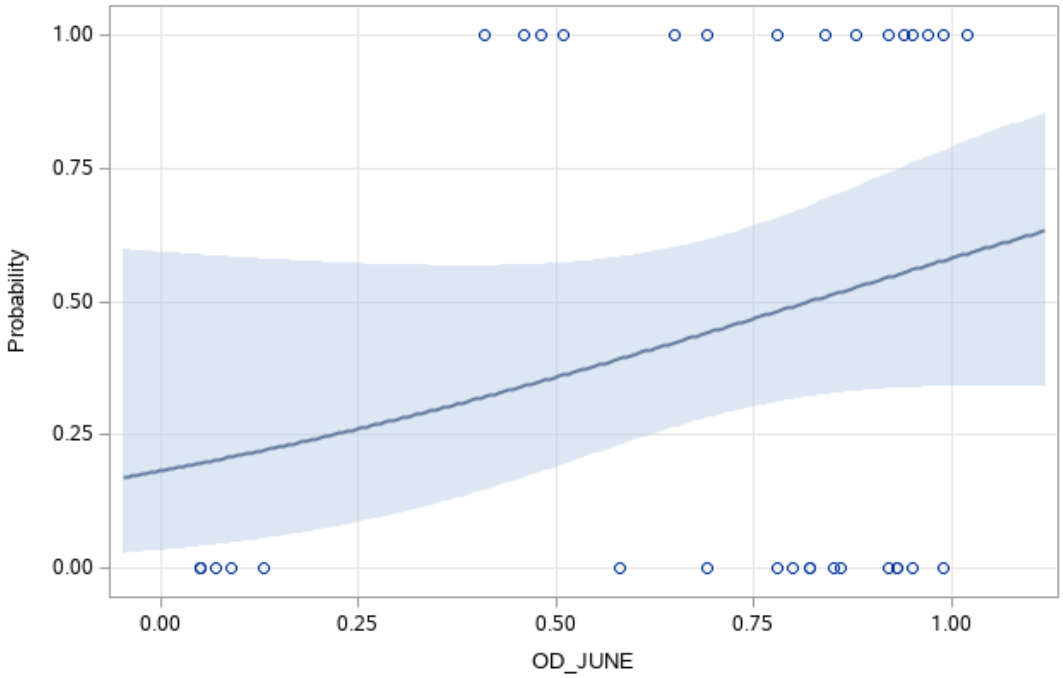


Figure 3. Probability of August Optical Densities of Pregnancy Specific Protein B to Predict Calving in the First 31 Days of the Calving Season

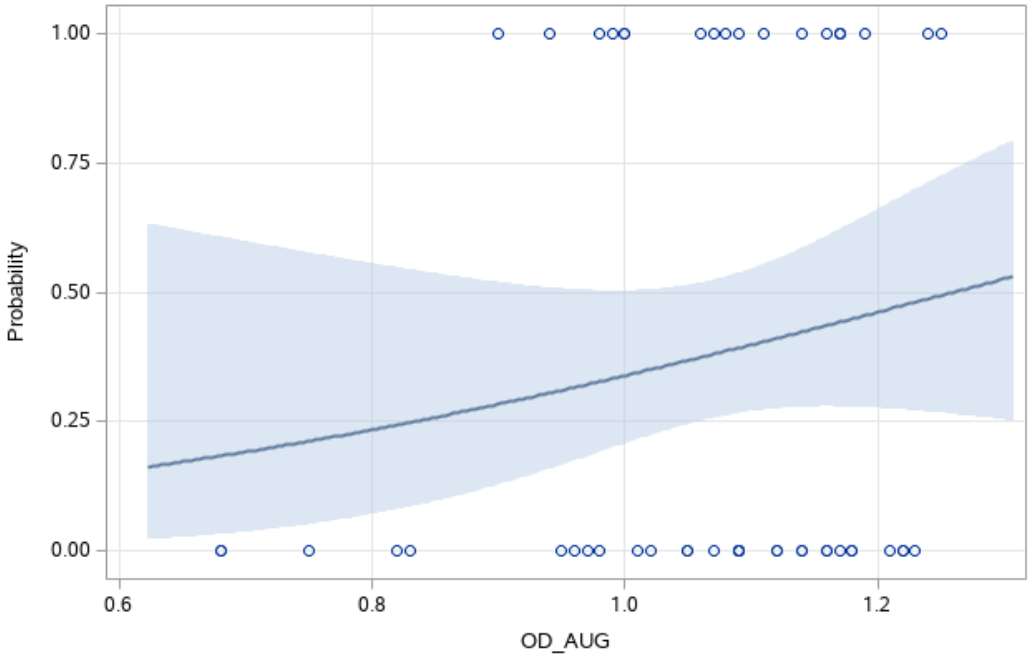
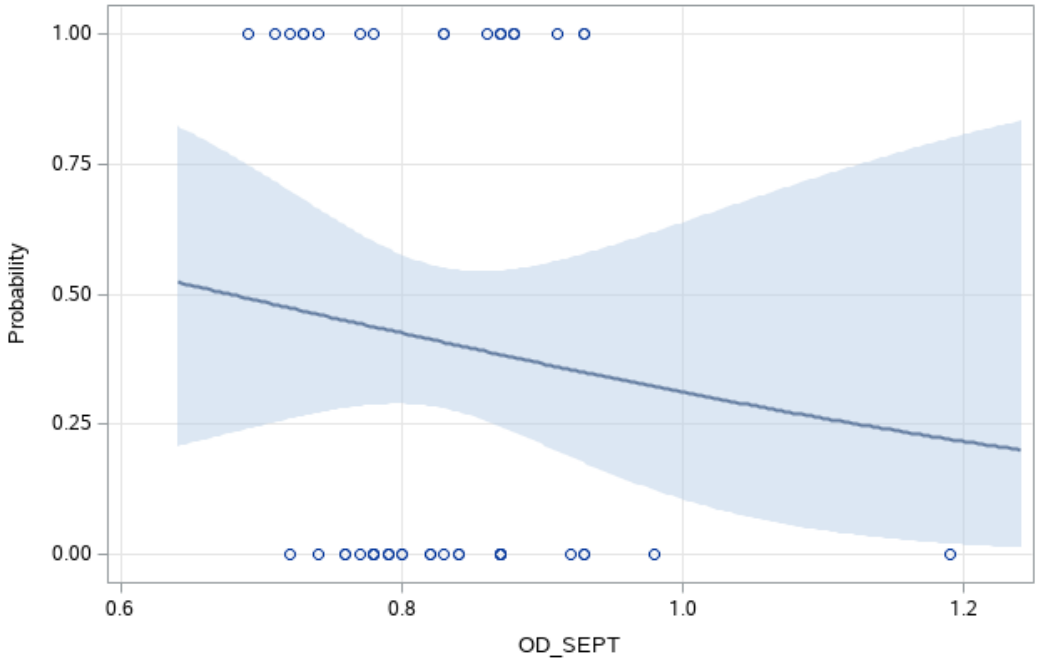


Figure 4. Probability of September Optical Densities of Pregnancy Specific Protein B to Predict Calving in the First 31 Days of the Calving Season



IMPLICATIONS

The data and results of this study provide useful information for the agricultural sector, more specifically for cattle operations. Reproductive efficiency is a trait that producers strive to carry amongst the cow herd to increase productivity. Being said, pregnancy diagnosis and maintenance are important factors for decision-making. The concentrations of PSPB can tell producers if a cow or heifer has bred. If the cow is not pregnant, decisions such as re-breeding or removing the animal can take place. Monitoring levels of PSPB using optical densities (OD) can also provide the same information in case of embryo loss. The results suggests that the first month of pregnancy for cows or heifers that bred early through AI can predict if they will calve within the first 31 days of the calving season. The subsequent months' OD show lower possibilities for prediction. The major points for the producer who is interested in using PSPB to monitor gestation in the cow herd is to use it for initial pregnancy diagnosis, test in the first month of pregnancy to record which cows are likely to calve first, and then test one more time at least 45 days after the bull is removed. This will allow the producer to more confidently know which cows remained not pregnant or lost the pregnancy for future reference when managing the reproduction protocols of the operation.

LITERATURE CITED

Ergene O., I. Darbaz, S. Sayiner, and S. Aslan. 2018. Comparison of bovine pregnancy associated glycoproteins (bPAG-serum and milk), bovine pregnancy specific protein B (bPSP-B) tests with each other and with transrectal ultrasonographic findings for early pregnancy diagnosis. Kafkas Univ Vet FakDerg, 24:497-502.

Love, D., M. Mefford, and J. Ramer. 2017. Validation of the Bio PRYN enzyme-linked immunosorbent assay for detection of pregnancy-specific protein-B (PSPB) and diagnosis of pregnancy in American bison (Bison bison). Reproduction in Domestic Animals 52:791-797.

Mapletoft, R.J., M.F. Martinez, M.G. Colazo and J.P. Kastelic. 2002. The use of controlled internal drug release devices for the regulation of bovine reproduction. J. Anim. Sci. 81:E28-E36.

Patel, O., S. Camous, N. Takenouchi, T. Takahashi, M. Hirako, N. Sasaki, and I. Domeki. 1998. Effect of stage of gestation and foetal number on plasma concentrations of a pregnancy serum protein (PSP-60) in cattle. Research in Veterinary Science 65:195-199.

Patel, O., I. Domeki, N. Sasaki, T. Takahashi, M. Hirako, R. Sasser, and P. Humblot. 1995. Effect of fetal mass, number and stage of gestation on pregnancy-specific protein B concentrations in the bovine. Theriogenology 44:827-833.

Pohler, K., M. Pereira, F. Lopes, J. Lawrence, D. Keisler, M. Smith, J. Vasconcelos, and J. Green. 2016. Circulating concentrations of bovine pregnancy-associated glycoproteins and late embryonic mortality in lactating dairy herds. J. Dairy Sci. 99:1584-1594.

Romano, J. E. and J. E. Larson. 2010. Accuracy of pregnancy specific protein-B test for early pregnancy diagnosis in dairy cattle. Theriogenology 74:932-939.

Sasser, R. G., C. A. Ruder, K. A. Ivani, J. E. Butler, and W. C. Hamilton. 1986. Detection of pregnancy by radioimmunoassay of a novel pregnancy- specific protein in serum of cows and a profile of serum concentrations during gestation. Biology of Reproduction 35:936-942.

Senger, P.L. 1997. Pathways to pregnancy and parturition. Redmond, OR: Current Conceptions. pg 236.

Scully, S., S. Butler, A. Kelly, A. Evans, P. Lonergan, and M. Crowe. 2014. Early pregnancy diagnosis on days 18 to 21 postinsemination using high-resolution imaging in lactating dairy cows. J. Dairy Sci. 97:3542-3557.

Wallace, J. M., R. P. Aitken, M. A. Cheyne, and P. Humblot. 1997. Pregnancy-specific protein B and progesterone concentrations in relation to nutritional regimen, placental mass and pregnancy outcome in growing adolescent ewes carrying singleton fetuses. Reproduction 109:53-58.

APPENDIX

ANGELO STATE UNIVERSITY
College of Graduate Studies & Research
Institutional Animal Care & Use Committee

February 25, 2019

Mike Salisbury, Professor
Agriculture
Angelo State University
ASU Station #10888
San Angelo, TX 76909

Your proposed project titled, "Pregnancy Specific Protein B as a predictor of gestation length in Angus cows and heifers" was reviewed by Angelo State University's Institutional Animal Care and Use Committee (IACUC) in accordance with the regulations set forth in the Animal Welfare Act and P.L. 99-158.

This protocol was approved for three years, effective February 25, 2019 and it expires three years from this date; however, an annual review and progress report form (www.angelo.edu/content/files/22583iacuc-annual-review-progressreport) for this project is due on August 15 of each year. If the study will continue beyond three years, you must submit a request for continuation before the current protocol expires.

The protocol number for your approved project is 2019-104. Please include this number in the subject line of in all future communications with the IACUC regarding the protocol.

Sincerely,

Chase Runyan, Ph.D.
Co-Chair, Institutional Animal Care and Use Committee

VITA

Candida Katalina Chairez was born and raised near the border, in the small town of Del Rio, Texas. She graduated from Del Rio High School in 2012. She received a B.S. in Animal Science from Angelo State University in May 2016 and also completed her career as a student-athlete for the track and field program. Candida has been employed at Southside Animal Hospital and Concho Valley Veterinary Emergency Association during her academic career.

Permanent Address:

Candida K. Chairez

306 Far Hills Dr.

Del Rio, Texas 78840