Research Journal of Animal Sciences 1 (2): 65-71, 2007

Predicting Pregnancy Status and Fetal Number in Time-Dated Pregnant Ewes Using Serum Progesterone and Ultrasound

[1]K. See, [1]A.J. Bailer, [2]E.J. Behnke, [3]R.S. Baker, [3]K.E. Clark and [4]P.T. Russell
[1]Department of Mathematics and Statistics, Center for Environmental Toxicology and Statistics, Miami University, Oxford, Ohio 45056, U.S.A
[2]Institute for Reproductive Health, 3805 Edwards Road, Cincinnati, Ohio 45209, U.S.A
[3]Divisions of Maternal-Fetal Medicine and Reproductive Endocrinology, Department of Obstetrics and Gynecology, College of Medicine, University of Cincinnati, 231 Albert Sabin Way, Cincinnati, OH 45267-0526, U.S.A
[4]Russell Farm, 4486 McKeever Road, Williamsburg, Ohio 45176, U.S.A

Abstract: In recent years, substantial interest has arisen in fetal imprinting of adult disease. An animal model utilized to study this phenomenon is the pregnant ewe and her offspring. Such studies require the use of pregnant ewes and a way of determining if the animal is carrying a singleton because fetal growth is significantly affected by the presence of twins. The prediction of pregnancy status was based on field ultrasound, Scanopreg (Ithaco, Ithaca, New York, model #738), 60 days after breeding and compared to pregnancy predictions based on a single progesterone value. Fetal number was determined at the time of delivery or at surgery. Non- pregnant ewes had a mean progesterone level (standard deviation) of 2.04 (1.25) ng mL^{-1}, singleton pregnancies were associated with a mean level of 5.44 (1.36) ng mL^{-1} and twins with a mean value of 7.14 (2.57) ng mL^{-1}. Classification of sheep pregnancy status and fetal number was examined by considering rates of correct classification and error rates associated with misclassification, along with Receiver Operating Characteristic (ROC) analysis. Results showed that both ultrasound and progesterone are reasonable screens of pregnancy status, but progesterone alone is not sufficient for differentiating between singleton and twin-bearing ewes.

Key words: Pregnancy status, progesterone level, screening test characteristics, sheep, ultrasound

INTRODUCTION

The pregnant ewe is a characterized and often used model for studying fetal growth and placental function. This is because the length of the ovine gestation provides ample time to study changes (lasting approximately 150 days), is durable under laboratory conditions, often has singleton pregnancies and the fetuses share similar morphological trends and growth characteristics with human fetuses. Most recently, the pregnant ewe and her offspring have become one of the main animal models utilized to study the phenomenon of fetal imprinting of adult disease and for developing fetal surgical techniques. These and other studies require the use of pregnant ewes that can be identified through some simple way of ascertaining both pregnancy and fetal number.

Both physical and chemical methods are available to determine pregnancy and fetal number of sheep. Serum progesterone would seem to have an advantage under many circumstances as it is a test both widely-available and relatively inexpensive. Its applicability, however, depends upon identifying circulating values that can distinguish between luteal phase and pregnancy levels and, during pregnancy, between levels produced by single or multiple lambs. This latter criterion, theoretically, would reflect placental mass which might be expected to increase with increased fetal number. In practice, though, blood progesterone concentrations have been proven to have but limited application (Gadsby *et al*., 1972; McPhee and Tiberghien, 1987; Müller *et al*., 2003).

The aim of this research was to determine and compare predictions of pregnancy status using two methods: Serum progesterone levels and ultrasound test. Serum progesterone was also examined to see if it could distinguish fetal number (i.e., singleton/twin status) in pregnant ewes. To evaluate the discriminating power of screening tests, we considered sensitivity, specificity, error rates and Receiver Operating Characteristic (ROC) curves.

Corresponding Author: K. See, Department of Mathematics and Statistics, Center for Environmental Toxicology and Statistics, Miami University, Oxford, Ohio 45056, U.S.A

MATERIALS AND METHODS

Animals: One hunderd and one Adult Suffolk or Suffolk-crossed ewes, 1-6 years old, were exposed to a proven male with a breeding harness during the September to November breeding season. Upon indication of mating (rump markings) the ewe was separated from the breeding pen, its animal number and date recorded and pastured away from further male contact. Beginning 50 days after marking at randomly chosen times, a blood sample was drawn from the jugular vein of each ewe and sent to the laboratory for a serum progesterone determination. All animals were maintained *ad libitum* on Rumilab feed and water for 2-3 weeks before sampling. In all cases, fetal numbers were determined at the time of normal delivery or upon inspection at surgery in the laboratory.

Progesterone: A blood sample was collected from the jugular vein of the 47 bred and pregnant ewes between gestational days 50-100 and up to 120 days in ewes that had not conceived or had not been bred. These samples were transported to the laboratory where the fresh serum, after clotting and centrifugation, was utilized for progesterone level determination, using a specific immunometric (chemiluminescence) assay (Immulite; DPC, Los Angeles, CA). The detection limit was 0.2 ng mL^{-1} and the interassay coefficient of variation was 7.7% for the period of analysis.

Ultrasound assessment of pregnancy: A Scanopreg unit (Ithaco, Ithaca, New York, model #738) was used. This unit reflects ultrasound waves from bodies of fluid, such as a gravid uterus or a full bladder.

Statistics: Screening tests, based on progesterone levels or ultrasound, were used to predict physiological status (here pregnancy or fetal number) by comparing the results of a diagnostic test to a cutoff to classify individuals into one of two categories. When evaluating the performance or discriminating power of screening tests, it is important to consider various measures, such as sensitivity, specificity, predicted value positive and ROC curves (Rosner, 2004; Dodd, 1978).

Measures of screening tests for pregnancy status: For measures of screening tests, we considered sensitivity, specificity, error rates and ROC curves. A good diagnostic test is one which has a high sensitivity (e.g., pregnancy test is positive when the ewe is truly pregnant) and high specificity (e.g., pregnancy test is negative when the ewe is truly not pregnant). In addition, a low false negative rate is important. Sensitivity and specificity are related with high sensitivity often paired with low specificity, resulting in a greater false positive rate (= 1-specificity) in this case. Comparing screening tests involves a balance of these traits. A screening test that yields continuous test measurements (e.g., progesterone levels) is usually performed by comparing an observed value or characteristic to a cutoff point (Table 1). A classification of positive or negative test is made based upon where the observed result lies with respect to this cutoff point. A contingency table such as Table 2 or 3 can be formed for different cutoff points. A screening test that produces a dichotomous outcome (e.g. ultrasound yields pregnant/non-pregnant prediction) can be compared directly to the true status using a table as well (Table 4). The properties of screening tests as shown in Table 5 can then be computed for each screening test considered.

The Receiver Operating Characteristic (ROC) curve: An ROC curve is a graphical representation of the trade off between the false negative and false positive rates for a range of cut off values (Fischer *et al*., 2003; Hanley and McNeil, 1982). Equivalently, the ROC curve gives a graphical representation of how well the test performs with respect to sensitivity and specificity. The plots show the false positive rate (=1-specificity) on the horizontal X axis and sensitivity (=1-false negative rate) on the vertical Y axis. In general, the more steeply the ROC curve climbs towards the upper left hand corner of the graph, the better the test. This means that sensitivity

Table 1: Numerical descriptive summary of serum progesterone level (ng mL^{-1})

Group	n	Mean (SD)	Min.	First quartile	Median	Third quartile	Max.
Non-Pregnant	54	2.04(1.25)	0.02	1.00	1.90	3.00	4.40
Singleton	30	5.44(1.36)	2.80	4.58	5.30	6.03	9.30
Twins	17	7.14(2.57)	3.40	4.90	7.00	9.45	12.6

Table 2: Cross tabulation of progesterone prediction results using a cutoff of 4.3(ng mL^{-1}) for the pregnant and non-pregnant ewes

	Pregnancy status		
	Pregnant	Non-pregnant	Test total
Progesterone ≥ 4.3 (ng mL^{-1})	40	4	44
Progesterone < 4.3 (ng mL^{-1})	7	50	57
Pregnancy status total	47	54	101

Table 3: Cross tabulation of progesterone prediction results using cutoff of 6.0(ng mL^{-1}) for the twins and singleton ewes

	Pregnancy status		
	Twins	Singleton	Test total
Progesterone ≥ 6.0 (ng mL^{-1})	11	8	19
Progesterone < 6.0 (ng mL^{-1})	6	22	28
Pregnancy status total	17	30	47

Table 4: Ultrasound test results with actual pregnancy status

	Pregnancy status		
	Pregnant	Non-pregnant	Test total
Ultrasound positive	58	8	66
Ultrasound negative	6	9	15
Pregnancy status total	64	17	81

Table 5: Comparisons of measures of two screening tests: Ultrasound test and serum progesterone prediction

Measures	Prediction based on Ultrasound test (%)	Prediction based on Progesterone prediction (%)
Sensitivity[1]	91	85
Specificity[2]	53	93
PVP[3]	88	91
PVN[4]	60	88
False positive[5]	47	7
False negative[6]	9	15

[1]Sensitivity = Pr(T+|D+) = The probability that a pregnancy test is positive given that the ewe truly is pregnant, where T+ = test result is postitive (or "high" test result) and D+ = actual pregnancy status is positive.
[2]Specificity = Pr(T-|D-) = the probability that a pregnancy test is negative given that the ewe is not pregnant, where T- = test result is negative (or "low" test result) and D- = actual pregnancy status is negative.
[3]Predictive Value Positive (PVP) = Pr(D+|T+) = the probability that a ewe is pregnant given a positive pregnancy test
[4]Predictive Value Negative (PVN) = Pr(D-|T-) = the probability that a ewe is not pregnant given a negative pregnancy test
[5]False Positive = 1-Specificity
[6]False Negative = 1-Sensitivity

is high and the false positive rate is low. The closer the ROC curve is to a diagonal, the less useful the test is at discriminating between positive and negative conditions. A more precise way of characterizing this closeness to the diagonal is simply to look at the area under the ROC curve, which can be calculated in many software packages including SAS PROC LOGISTIC (SAS Institute, 2000). A very rough index, or interpretation, of the areas under the ROC curve (AUC) was suggested with AUC in the range 0.50-0.75 = fair test performance; 0.75-0.92 = good; 0.92-0.97 = very good; 0.97-1.00 = excellent (Choi, 1998). In general, the closer the area is to 0.5, the worse the test and the closer it is to 1.0, the better the test.

RESULTS

Progesterone based prediction results of pregnant and non-pregnant ewes: Figure 1 shows distribution of progesterone levels, as displayed in a relative frequency histogram, with density estimates, for non-pregnant, singleton and twin-bearing ewes. Table 1 summarizes the basic descriptive statistics of progesterone levels for the 101 ewes used in this study. In sum, the progesterone level of the pregnant ewes is greater (mean ± SD: 5.441.36 ng mL^{-1} for singleton and 7.142.57 ng mL^{-1} for twins) than that of the non-pregnant ewes (2.041.25 ng mL^{-1}).

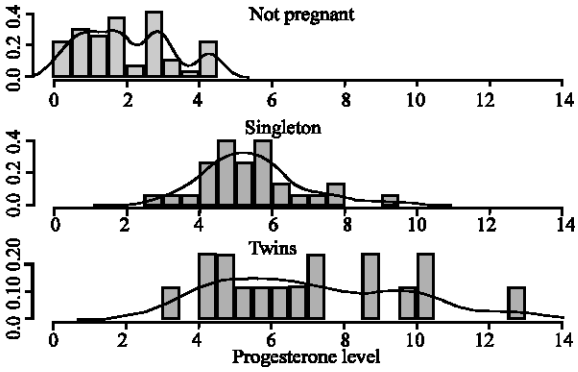

Fig. 1: Relative frequency histograms of progesterone levels (ng mL^{-1}) along with a superimposed density estimate displayed by pregnancy status

Among the pregnant ewes, the median progesterone level for the ewes carrying twins is 7.00 ng mL^{-1}, which is greater than that of ewes with a single fetus (5.30 ng mL^{-1}). But, there is considerable overlap in the progesterone distribution for the two pregnant groups due to large variability present (Fig. 1) in the progesterone levels in the ewes carrying twins.

ROC analysis of progesterone cut-points: Figure 2 shows ROC curves for the progesterone-based predictions of pregnancy status (a) and for the prediction of singletons vs. twins (b). In particular, Figure 2a provides evidence that progesterone-based predictions can be used to discriminate between pregnant ewes and non-pregnant ewes. The curve moves rapidly towards the upper left hand corner of the graph. This indicates a greater initial likelihood of obtaining a true positive result. Only later, as we start to encounter more false positives, will the curve ease off and become horizontal (corresponding to the use of smaller cut-points). Figure 2b shows an ROC curve for using progesterone level to discriminate between fetal numbers. This curve is somewhat closer to diagonal, implying that each gain in sensitivity is balanced by a similar loss in specificity and vice versa. Thus, the progesterone test does not appear to be as useful in distinguishing between ewes carrying twins and singletons. The estimated AUC using SAS PROC LOGISTIC in Fig. 2a is 0.997 (or 99.7%) and the estimated AUC in Fig. 2b is 0.693 (or 69.3%). This suggests that a diagnostic test defined by a progesterone cutoff point provides an excellent diagnostic procedure for determining pregnancy status (pregnant vs. non-pregnant); however, it provides a poor-to-fair diagnostic procedure for predicting twin vs. singleton status.

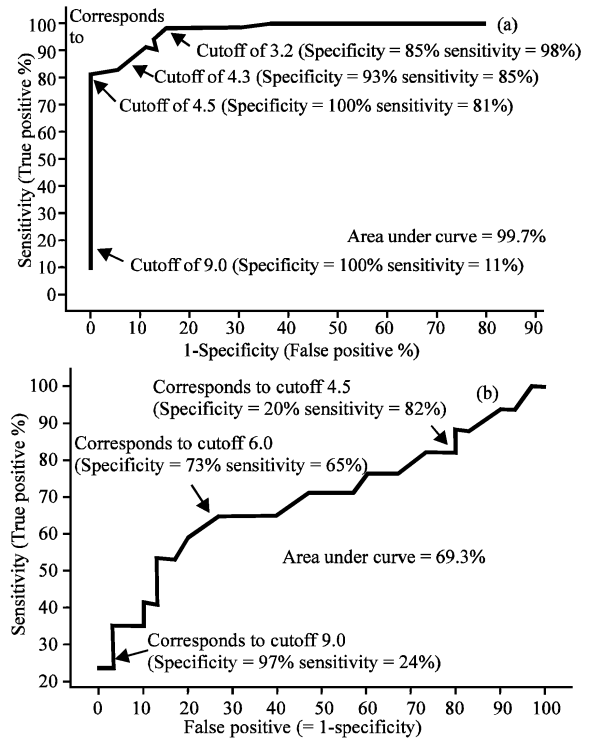

Fig. 2: ROC curve for pregnant and non-pregnant group (a) and twins and singleton group (b) prediction based upon progesterone levels with the estimated area under the curve 0.997 and 0.693, respectively

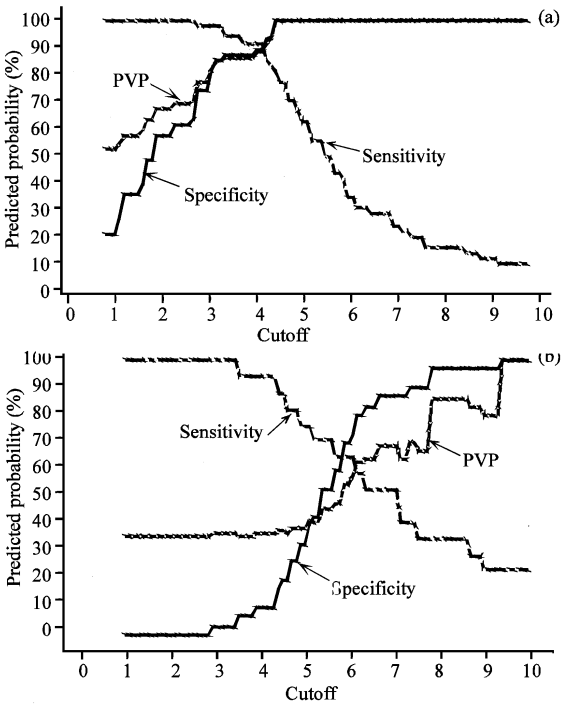

Fig. 3: Progesterone prediction results on sensitivity, specificity and Predictive Value Positive (PVP) for various cutoff points for pregnant vs non-pregnant ewes (a) and ewes carrying twins and singleton (b)

Selection of cutoff points: The cutoff point which yields the best combination of values for the properties is chosen as the reference point to use when conducting the test. Figure 3 depicts the graphical display of the results of the three important properties of a screening test: sensitivity, specificity and Predictive Value Positive (PVP) in percent, for cutoff points ranging from 0.8-10 incremented by 0.1. Recall that a test with high sensitivity, specificity and PVP is desirable. Figure 3a contains the prediction results for pregnant vs. non-pregnant comparisons and the best combination cutoff value for these three properties appears to be approximately 4.3. In Figure 3b, for the twins vs. singleton comparisons, the best combination cutoff value for the three properties appears to be around progesterone level 6.0.

Table 2 shows a classification of the results of a progesterone prediction test for the pregnant vs. non-pregnant ewes, based on the cutoff value 4.3 ng mL^{-1}. The use of this reference point is justified in the following section. If the progesterone level was greater than or equal to the cutoff point of 4.3 ng mL^{-1}, then the test was declared "positive" for pregnancy. If the progesterone level was less than the cutoff point 4.3 ng mL^{-1}, then the test was considered "negative" for pregnancy.

Based on the progesterone test results in Table 2, the following properties distinguished between pregnant and non-pregnant ewes. Sensitivity was 40/(40+7)= 85% and specificity was 50/(4+50)=93%. This means that about 85% of pregnant ewes demonstrated progesterone levels greater than or equal to 4.3 ng mL^{-1} and about 93% of non-pregnant ewes had progesterone levels less than 4.3 ng mL^{-1}. The false positive result was 4/(4+50)=7% while the false negative result was 7/(40+7)=15%. Thus, about 7% of non-pregnant ewes had progesterone levels greater than 4.3 ng mL^{-1} and about 15% of pregnant ewes showed progesterone levels lower than 4.3 ng mL^{-1}. The PVP was 40/(40+4) = 91%, meaning that among ewes with the greater progesterone levels (4.3ng mL^{-1}), about 91% were pregnant. The Predictive Value Negative (PVN) was 50/(7+50) = 88%, which implies that among the ewes with lower progesterone levels (<4.3 ng mL^{-1}), about 88% were not pregnant.

Progesterone-based prediction of twins and singleton: From Table 3, just as we obtained with the results in Table2, the following properties were found to distinguish between twins and singleton ewes. Sensitivity is 11/(11+6)= 65% and specificity is 22/(8+22) = 73%. This means that about 65% of ewes carrying twins will have progesterone level greater than or equal to 6.0 ng

mL^{-1} and about 73% of ewes carrying singletons will have progesterone levels less than 6.0 ng mL^{-1}. False positive (=1-specificity) is 27% and false negative (=1-sensitivity) is 35%. Thus, about 27% of ewes with singletons will have progesterone levels greater than or equal to 6.0ng mL^{-1} and about 35% of ewes with twins will have progesterone levels less than 6.0ng mL^{-1}. The PVP is 11/(11+8)=58%; thus, of the ewes with progesterone levels greater than or equal to 6.0ng mL^{-1}, about 58% are carrying twins. The PVN is 22/(6+22)=79% implying that among the ewes with progesterone levels less than 6.0ng mL^{-1}, about 79% are carrying a singleton.

Ultrasound-based prediction of pregnant vs. Non-pregnant ewes: Table 4 contains results of ultrasound tests that have confirmed outcomes over a 4-year period, beginning in 2000, for pregnant and non-pregnant ewes. Based on the ultrasound test results in Table 4, we obtain the following properties to distinguish between pregnant and non-pregnant ewes, a sensitivity of 91% and a specificity of 53%. Thus about 91% of pregnant ewes had a positive ultrasound test result and about 53% of non-pregnant ewes had a negative ultrasound test. This gave a false positive rate of 47% while the false negative rate was 9%. Hence, about 47% of non-pregnant ewes will have positive ultrasound tests and about 9% of pregnant ewes will have negative ultrasound results. The PVP is 88%, indicating that among the ewes with positive ultrasound results, about 88% were actually pregnant. Alternatively, a PVN of 60% implies that among the ewes with negative ultrasound results, about 60% were not pregnant.

Comparison of ultrasound test vs. progesterone predictions on pregnant and non-pregnant ewes: Table 5 compares the properties of the Scanopreg ultrasound test in our study and progesterone predictions. The progesterone test has about 40% greater specificity (93 vs. 53%), or equivalently, 40% smaller false positive rate. In fact, the test provides a smaller false positive rate across a reasonable range of cutoff values (less than 10% false positive rates for progesterone levels greater than or equal to 4.3ng mL^{-1}). Also, the progesterone test gives greater prediction values: 3% greater in PVP (91 vs. 88%) and 28% greater in PVN (88 vs. 60%). However, when compared with the progesterone prediction outcomes based on the progesterone level 4.3ng mL^{-1}, the ultrasound test gives about 6% greater sensitivity (91 vs. 85%) than serum progesterone prediction and about 6% lower false negative rates (9 vs. 15%). This implies that ultrasound tests tend to give greater true positive fraction and lower false negative fraction when compared to the progesterone predictions. Thus, the ultrasound test is slightly more responsive in identifying pregnant ewes at the cost of falsely declaring pregnant a large fraction of non-pregnant ewes.

DISCUSSION

Circulating levels of progesterone in the pregnant ewe originate primarily from the ovary during the first trimester of the ovine pregnancy (days 0-48) and from the placenta thereafter. Ovariectomy after day 50 of gestation does not terminate pregnancy (Casida and Warwick, 1945; Denamur and Martinet, 1955) and placental progesterone production rates appear to greatly exceed ovarian production in late pregnancy (Fylling, 1970; Lynzell and Heap, 1968). Progesterone provides vital hormonal support to the endometrium and other processes necessary for a viable pregnancy (Denamur and Martinet, 1955; Spencer and Fuller, 2002).

Throughout the first two thirds of pregnancy, progesterone levels increase gradually, finally demonstrating a dramatic rise about four weeks before parturition followed by a precipitous decline about two weeks later (Nalbandov, 1976). During the "placental" phase of gestation, progesterone levels tend to be greater in ewes carrying multiple fetuses although considerable variability has been noted (Bassett *et al*., 1969; Emady *et al*., 1974; Gadsby *et al*., 1972; Stabenfeldt *et al*., 1972). This difference in circulating progesterone could theoretically provide an opportunity to identify the number of fetuses carried by a ewe based solely on measured blood progesterone levels. This assumes that there would be a greater requirement for progesterone with an increase in the products of conception. However, the review by Spencer and Bazer (2002) describes multiple interactions and vital functions for progesterone during the course of a pregnancy that could preclude a requirement for progesterone above base-line levels but adequate to successfully maintain a pregnancy. Hence, from this line of reasoning, there need not be incremental increases in circulating progesterone to accommodate an increase in the number of lambs carried by the ewe. The present study of 101 ewes demonstrates that a single progesterone value offers only poor to fair success in distinguishing twin from singleton pregnancies in a flock under field conditions.

Progesterone has also been used to diagnose pregnancy (Gadsby *et al*., 1972; McPhee and Tiberghien, 1987; Robertson and Sarda, 1971). During the breeding season, the ewe cycles about every 17 days with progesterone peaking around day 10 (McPhee and Tiberghien, 1987; Stabenfeldt *et al*., 1969; Xia *et al*., 2003). During that time, circulating progesterone values can vary from 0.1 ng mL^{-1} to those in excess of 5 ng mL^{-1}.

Because pregnant ewes maintain progesterone at or slightly below the luteal high in early gestation with an increasing upward trend as gestation advances (Stabenfeldt *et al*., 1972), a systematic reassessment of progesterone at day 10 can identify pregnant ewes with >90% reliability (Robertson and Sarda, 1971). But pregnancy can also be diagnosed using a single progesterone value. The simplicity and accuracy of this approach often has considerable advantages over many of the more elaborate and expensive ultrasound methods for diagnosing pregnancies in sheep (Grant and Warren, 1980; Taverne *et al*., 1985; Watt *et al*., 1984).

In sum, progesterone predictions work very well for the pregnant and non-pregnant group distinctions, for twins vs. singleton prediction, the progesterone test provides only minimal discrimination and overall, when comparing the ultrasound test to the progesterone test, it appears that the progesterone test is somewhat superior in distinguishing between the pregnant and non-pregnant groups (based on the cutoff 4.3ng mL^{-1}). Specifically, the progesterone test gives greater specificity and greater prediction values. In addition, the progesterone test provides a smaller false positive rate across a reasonable range of cutoff values (less than 10% false positive rates for progesterone levels greater than or equal to 4.3ng mL^{-1}). However, the ultrasound test gives about 6% greater sensitivity than serum progesterone prediction when compared with the progesterone prediction based on a cutoff of 4.3ng mL^{+} and about 5% lower false negative rate (1-Sensitivity). This implies that ultrasound tests tend to give greater true positive rate and lower false negative rate when compared to the progesterone predictions. One important caveat for this analysis is that the progesterone cutoff value (e.g., 4.3 ng mL^{-1}) is an estimated quantity that may be relevant for this particular breed of sheep. The methods and discussion presented herein can be generalized to other screening test contexts. Thus, while progesterone-based predictions do not provide total assurance of the number of fetuses *in utero*, they do provide a screening test with relatively low false positive and false negative error rates relative to ultrasound screening offering assistance to the supplier and investigator with reasonable guidelines in choosing appropriate ewes for studies.

CONCLUSION

Progesterone levels can be used for differentiating between pregnant and non-pregnant ewes. Pregnancy tests based on progesterone levels exhibit greater specification and lower false positive rates when compared to pregnancy tests based on ultrasounds. Finally, progesterone levels provided little discrimination between fetal numbers for pregnant ewes. Thus, additional variables would need to be considered if an accurate screening test of fetal number was desired.

ACKNOWLEDGEMENT

The authors wish to thank Mr. J. M. Doss for his expert technical assistance. The authors also wish to thank Mr. Michael Hughes for his contribution to the discussions.

REFERENCES

Bassett, J.M., T.J. Onborrow, I.D. Smith and G.D. Thorburn, 1969. The concentration of progesterone in the peripheral plasma of the pregnant ewe. J. Endocr., 45: 449-457.

Casida, L.E. and E.J. Warwick, 1945. The necessity of the corpus luteum for maintenance of pregnancy in the ewe. J. Anim. Sci., 4: 34-36.

Choi, B.C., 1998. Slopes of a receiver operating characteristic curve and likelihood ratios for a diagnostic test. Am. J. Epidemiol., 148: 1127-1132.

Denamur, R. and J. Martinet, 1955. Effets de l'ovariectomie chez la brebis pendant la gestation. C. r. Seanc. Soc. Biol., 149: 2105-2107.

Dodd, K., 1978. Estimation of the sensitivity, specificity and predictive value of the intradermal tuberculin test. Irish Vet. J., 32: 87-89.

Emady, M., J.C. Hadley, D.E. Noakes and G.H. Arthur, 1974. Progesterone level in the peripheral blood of pregnant ewes. Vet. Rec., 75: 985-960.

Fischer, J.E., L.M. Bachmann and R. Jaeschke, 2003. A readers' guide to the interpretation of diagnostic test properties: Clinical example of sepsis. Intensive Care Med., 29: 1043-1051.

Fylling, P., 1970. The effect of pregnancy, ovariectomy and parturition on plasma progesterone level in sheep. Acta Endocr. (Kbh), 65: 273-283.

Gadsby, J.E., R.B. Heap, D.G. Powell and D.E. Walters, 1972. Diagnosis of pregnancy and of the number of foetuses in sheep from plasma progesterone concentrations. Vet. Rec., 90: 339-342.

Grant, C.G. and R.R. Warren, 1980. Sheep breeding program for the year-round production of timed pregnancies for experimental foetal surgery. Laboratory Anim., 14: 317-321.

Hanley, J.A. and B.J. McNeil, 1982. The meaning and use of the area under a receiver operating characteristic (roc) curve. Diagnostic Radiol., 143: 29-36.

Lynzell, J. L. and R. B. Heap. 1968. A comparison of progesterone metabolism in the pregnant sheep and goat: Sources of production and an estimation of uptake by some target organs. J. Endocr., 41: 433-438.

McPhee, I.M. and M.P. Tiberghien, 1987. Assessment of pregnancy in sheep by analysis of plasma progesterone using an amplified enzyme immunoassay technique. Vet. Rec., 121: 63-65.

Müller, T., H. Schubert and M. Schwab, 2003. Early prediction of fetal numbers in sheep based on peripheral plasma progesterone concentrations and season of the year. Vet. Rec., 152: 137-138.

Nalbandov, A.V., 1976. Reproductive physiology of mammals and birds. (3rd Edn.), W.H. Freeman and Company, San Francisco.

Robertson, H.A. and I.R. Sarda, 1971. A very early pregnancy test for mammals: Its application to the cow, ewe and sow. J. Endocrinol., 49: 407-419.

Rosner, B., 2004. Fundamentals of biostatistics. (6th Edn.), Duxbury.

SAS, 2000. SAS Proc Logistic Procedure. SAS Institute Inc., SAS Campus Drive, Cary, North Carolina.

Spencer, T.E. and F.W. Bazer, 2002. Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. Frontiers in Biosci., 7: 1879-1898.

Stabenfeldt, G.H., M. Drost and C.E. Franti, 1972. Peripheral plasma progesterone levels in the ewe during pregnancy and parturition. Endocrinology, 90: 144-150.

Stabenfeldt, G.H., J.A. Holt and L.L. Ewing, 1969. Peripheral plasma progesterone levels during the ovine estrous cycle. Endocrinology, 85: 11-15.

Taverne, M.A.M., M.C. Lavoir, R. Van Oord and G. C. Van der Weyden, 1985. Accuracy of pregnancy diagnosis and prediction of foetal numbers in sheep with linear-array real-time ultrasound scanning, Vet. Quarterly, 7: 256-263.

Watt, B.R., G.A. Anderson and I.P. Campbell, 1984. A comparison of six methods used for detecting pregnancy in sheep. Aus. Vet. J., 61: 377-382.

Xia, Y., T. O'Shea, R. Murison and J.R. McFarlane, 2003. Concentrations of progesterone, follistatin and follicle-stimulating hormone in peripheral plasma across the estrous cycle and pregnancy in merino ewes that are homozygous or noncarriers of the booroola gene. Biol. Reprod., 69: 1079-1084.