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Embryonic Vesicle Depth using Transabdominal Ultrasound Early in Gestation as a Predicter of Gestational Age in Sheep

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ABSTRACT

Ultrasonography is commonly used by producers to determine pregnancy in their flocks (Scott 2012; Jones et al., 2016). Transabdominal ultrasonography is a simple, non-invasive method; however, it is not frequently used for early pregnancy detection in sheep. The aims of the current study were to determine whether pregnancy detection through transabdominal ultrasound is viable as early as day 25 of gestation and if a single measure of the embryonic vesicle could predict gestational age. Transabdominal ultrasound was performed on a total of 11 ewes at days 25, 27, 29, 31, 33 and 60 of gestation. Pregnancy diagnosis and accuracy of pregnancy detection were determined, and ultrasonic measurement of embryonic vesicle depth (EVD) was recorded for each ultrasound time period. Pregnancy may be determined at day 25 (accuracy = 45%); however, accuracy of pregnancy detection increased until day 31 (100%). A significant correlation between gestational age and EVD was identified ($P < 0.001$; r = 0.791). A regression analysis of gestational age (days since conception) on EVD resulted in a significant correlation of determination of 0.675 ($P \leq 0.001$). This resulted in a quadratic predictive equation to estimate gestational age using EVD ($y = 9.32 + 9.10 * EVD - 0.38 * EVD^2$). The results of the present study show that early pregnancy detection is possible in sheep; however, accuracy of pregnancy detection is reduced prior to day 31. Embryonic vesicle depth was able to predict gestational age with a simple quadratic equation. The number of fetuses present during ultrasonography was not counted in the present pilot study and may impact EVD and thus the estimation of gestational age. Future work in this area is warranted to investigate embryonic vesicle depth and the number of fetuses present during ultrasonography to predict gestational age.

Keywords: embryonic vesicle, gestational age, pregnancy diagnosis, sheep, transabdominal ultrasound

INTRODUCTION

Early pregnancy detection in ewes allows producers to efficiently provide adequate nutrients while minimizing production costs (NRC, 2007). To maximize reproductive efficiency, early pregnancy detection and estimation of gestational age is required (Haibel, 1990). Transabdominal ultrasonography, used for early pregnancy detection, is typically performed at a transducer frequency of 3.5 MHz and has been shown to have accuracies of 90% and 100% by days 31 and 45 of gestation, respectively (Jones et al., 2016). Determination of parturition date or due date is another key aspect of management in livestock. While fetal measurements have been shown to accurately predict gestational age (Gonzalez de Bulnes et al., 1998; Petrujkic et al., 2016), less research has evaluated the use of embryonic vesicle measurements. The objectives of the present pilot study were to determine if transabdominal ultrasonography could be used to accurately diagnose pregnancy in ewes between days 25 and 33 of gestation and determine if a single measurement of embryonic vesicle depth (EVD) could be utilized to predict gestational age.

MATERIALS AND METHODS

Animals. All procedures were approved by the Institutional Animal Care and Use Committee at Illinois State University (2019-1093). Eleven crossbred ewes (Dorset x Suffolk) were used for this study with an average age of 3.5 ± 0.8 years (average parity 2.5) and an average initial weight of 77.8 ± 7.1 kg (mean ± standard deviation). Ewes were synchronized using a thirteen-day insertion of Eazi-Breed CIDR (Zoetis, Parsippany, NJ). Eleven days after CIDR insertion, ewes were administered 263 mcg cloprostenol sodium (Estrumate; Merck Animal Health, Madison, NJ). At CIDR removal (d 13), ewes were administered 240 IU of equine chorionic gonadotropin and 120 IU of human chorionic gonadotropin (PG600; Merck Animal Health, Madison, NJ). One day post CIDR removal, mature rams were placed with the ewes for natural breeding.

Experimental Procedure. Transabdominal ultrasonography (Mindray Z6 and Mindray 3C5P Convex Transducer, Mindray North America, Mahwah, NJ) was performed on d 25, 27, 29, 31, 33, and 60 following ram introductions by three ultrasound technicians at a transducer frequency of 3.5 MHz. Ultrasound technicians were assigned to 3-4 ewes to perform the on-farm ultrasonography, as well as the subsequent ultrasonic measurements. Ultrasonography performed on day 25 post ram introduction was assumed to be approximately day 25 of gestation. For ultrasonography, ewes were restrained in a sheep head gate, the ultrasound transducer was lubricated and placed on the wool free section of the abdomen on the ewes' right side. On each ewe, a frontal cross-section image of the reproductive tract was saved for

later analysis.

Parameters evaluated included determination of pregnancy (yes or no), confidence of pregnancy determination (scale of 1 to 5; 1 = not confident and 5 = very confident), and embryonic vesicle depth (EVD). Additionally, accuracy of pregnancy detection was calculated as previously described by Jones et al. (2016). Embryonic vesicle depth measurements were performed using integrated software of the Mindray Z6 ultrasound. These were measured in the absence of the other ultrasound technicians. This measurement was chosen to minimize the effect of elongation of the embryonic sac during development. Ewes were subsequently followed through parturition where lambing date and number of lambs born were recorded.

Statisical Analysis. For EVD and confidence of pregnancy detection, an analysis of variance was performed using the mixed procedure of SAS (v9.4; SAS Institute, Cary, NC). The main effect for these parameters was gestational age. The model utilized repeated measures by ewe identification with number of lambs born and ultrasound technician included as covariates, when appropriate. For pairwise comparisons, the Tukey-Kramer adjustment was utilized. A regression analysis was performed using the surveyreg procedure in SAS where the dependent variable was gestational age, the independent variables were EVD and EVD^2, and the model was clustered by ewe identification. Embryonic vesicle depth squared was used in the model to improve the model as growth of embryonic vesicles is not linear. The quadratic model better fit the data from the present trial. Number of lambs was not included in the regression analysis, as they were determined after parturition not during ultrasonography. With alpha set at 0.05 and 0.01, this regression model yielded post-hoc power analyses of 0.95 and 0.73, respectively. For all statistical analyses, statistical differences were determined when $P \leq 0.05$.

RESULTS

Accuracy of pregnancy detection, a measure of correctly diagnosing both pregnant and non-pregnant females, was 45% on day 25, 64% on day 27, 82% on day 29, and 100% on days 31, 33 and 60, respectively (Table 1). Confidence of pregnancy detection was lower at d 25 compared to d 29, 33 and 60 ($P \leq 0.040$), tended to be lower at d 25 compared to 31 ($P = 0.071$), and was lower at d 27 compared to day 60 ($P = 0.040$; Table 1).

Gestational age had a significant effect on EVD ($P < 0.001$; Table 1) where d 60 was significantly greater than days 25-33 ($P < 0.001$) but did not differ between days 25-33 ($P \geq 0.107$). Gestational age and EVD were highly correlated in the present study ($P < 0.001$; Pearson's correlation coefficient = 0.791). Due to this strong correlation, a regression analysis was performed to predict gestational age using EVD. This resulted in a statistically significant regression model ($P < 0.001$; coefficient of determination = 0.675), where the intercept, EVD, and EVD^2 were also significant ($P \leq 0.007$; Table 2). The predictive equation for gestational age on EVD was: Gestational Age = 9.32 + 9.10 * EVD – 0.38 * EVD^2. As EVD increases, gestational age increases at a decreasing rate.

DISCUSSION

The present study demonstrated that early pregnancy detection using transabdominal ultrasonography is a viable option for producers in the sheep industry. Previous studies have reported pregnancy detection accuracies similar to those in the present study at comparable time points, suggesting that pregnancy detection as early as d 31 of gestation is feasible using transabdominal (Ganaie et al., 2009; Jones et al., 2016) and transrectal ultrasonography (Ali and Hayder, 2007). Pregnancy detection earlier than d 31 is possible; however, caution should be utilized due to the increased incidence of false negative diagnoses. Jones et al. (2016) similarly warned of the use of transabdominal ultrasonography prior to d 31 of gestation. The increased accuracy and confidence over time in the present study is likely a result of increased embryonic vesicle size and visual presence of concrete indications of pregnancy such as the fetus and placentomes (Ganaie et al., 2009; Jones et al., 2016). Moreover, technician training is critical in determination of pregnancy and may have influenced the confidence of pregnancy detection in the present study. However, the use of three inexperienced technicians resulted in 100% accuracy of pregnancy detection by d 31 of gestation. Jones et al. (2016), utilized a single, qualified technician to perform all ultrasonic measurements resulting in similar accuracies at d 30 (87.5%) and 33 (96.7%).

Measurements of EVD have been previously reported using transrectal ultrasonography in sheep at days 23

Table 1. Accuracy and sensitivity of pregnancy diagnosis using transabdominal ultrasonography in sheep.

	Day							
	25	27	29	31	33	60	SE	*P* value
Confidence of Detection[1]	3.83^{a}	4.27^{ab}	4.81^{bc}	4.80^{abc}	5.07^{bc}	5.25^{c}	0.28	0.003
Embryonic Vesicle Depth (cm)	1.65^{a}	2.61^{a}	2.89^{a}	2.70^{a}	3.56^{a}	7.20^{b}	0.59	< 0.001
True Positive (n)	5	7	9	11	11	11		
False Negative (n)	6	4	2	0	0	0		
Accuracy (%)[2]	45	64	82	100	100	100		

[1]Confidence of pregnancy detection on a scale of 1-5, where 1 = lack of confidence and 5 = very confident. ^{abc}Columns with differing letter are different ($P < 0.05$).
[2]Accuracy = [True Positive + True Negative]/[True Positive + True Negative + False Positive + False Negative]; Jones et al., 2016

Table 2. Regression parameter estimates of gestational age on embryonic vesicle depth, clustered by ewe ID ($n = 66$; Model *P* value < 0.001).

Variable	Estimate	SE	*P* value
Intercept	9.32	2.77	0.007
EVD[1]	9.10	1.37	< 0.001
EVD*EVD	-0.38	0.10	0.003
R^2	0.68		

[1]Measurements collected on days 25, 27, 29, 31, 33 and 60 of gestation.

(Petrujkić et al., 2016) and 30 of gestation (Buckrell et al., 1986). Petrujkic et al. (2016) demonstrated that gestational age can be accurately estimated between d 23 and d 38 of gestation by measuring EVD only when using transrectal ultrasonography ($R^2 = 0.926$, $P < 0.05$). In contrast to the present study, Petrujkic et al. (2016) reported that the use of transabdominal ultrasonography resulted in a non-significant regression analysis of gestational age on embryonic vesicle depth. The regression analysis by Petrujkic et al. (2016) utilized four time points whereas the regression in the present study utilized six. While EVD explained 68% of the variation in gestational age in the present study regression analysis, the number of fetuses were not included and may account for additional variation in the prediction of gestational age. To test the predictive equation from the present study, a single ultrasonic measurement of EVD from 9 non-research ewes was utilized in the predictive equation to determine gestational age which was then subtracted from 148 (assumed gestation length) to determine the number of days until lambing. Number of days until lambing was then utilized to calculate the predicted ewe due dates based on the date ultrasonography occured. Actual lambing dates averaged 1.9 ± 2.66 days (mean $\pm$ standard deviation) after the predicted due date. This illustrates the functionality of the predictive equation from the present study. Future studies should investigate the effect of gestational age on EVD and number of fetuses present during ultrasonography to generate a more accurate predictive equation.

SUMMARY

The present study demonstrated that pregnancy detection between days 25 through 29 of gestation were possible; however, accuracy of pregnancy diagnoses were 45% and 82%, respectively. Pregnancy diagnoses at day 31 of gestation resulted in an accuracy of 100%, suggesting that ultrasonography after day 30 of pregnancy is a viable option for producers. Additionally, a predictive equation was generated that can predict gestational age and subsequently predict ewe due date based on a single measure of embryonic vesicle depth. However, the predictive equation does not factor in number of fetuses present during ultrasonography and thus future research is needed to create a more robust predictive equation.

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