Pregnancy diagnosis in small ruminants

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Abstract

Pregnancy diagnosis in small ruminant species is crucial for the management of sheep and goats, particularly in commercial settings. There are many options for pregnancy diagnosis in these species, including ultrasonography and blood-based assays to detect pregnancy-associated glycoproteins. The method used often depends on the availability of veterinary services to perform pregnancy diagnosis and the management goals of the herd or flock. Regardless of the method chosen, it should be economical for the producer, easily performed in field conditions, and highly accurate. Additional information on gestational age and fetal number allows for improved nutritional management of the herd or flock to ensure adequate nutrition in the periparturient period to prevent metabolic disease such as pregnancy toxemia. Providing these diagnostic services can be an important source of revenue for the veterinarian while providing invaluable information to aid small ruminant producers in improving profitability in their herds and flocks.

Key words: pregnancy diagnosis, pregnancy-associated glycoproteins (PAG), ultrasonography, small ruminants

Introduction

Pregnancy diagnosis in livestock species is a critical component to herd management. In small ruminant species, opportunities to diagnose pregnancy may be limited by the availability of practitioners to perform the procedure, financial limitations of the clients, or lack of facilities for adequate restraint and handling. However, despite these challenges or potential barriers to implementation, pregnancy diagnosis can improve herd and flock management of sheep and goats. Early pregnancy diagnosis allows practitioners and producers to make rebreeding decisions for open females and culling decisions for females that may be subfertile or infertile. Additionally, determination of pregnancy status can improve economic returns in the flock or herd by eliminating the feeding of open ewes or does during the winter months when pasture is not as plentiful. If efforts to diagnose pregnancy in the does and ewes reveals suboptimal reproductive performance, it is important to remember to evaluate the ram or buck via a complete breeding soundness evaluation including examination for physical soundness, semen motility and morphology.

There are several methods for diagnosing pregnancy in small ruminants. The simplest, but least reliable or efficient, include observation for failure to return to estrus or waiting for lambing or kidding season. During the breeding season, sheep will cycle every 17 days and goats will show signs of estrus every 21 days. Failure to return to estrus may indicate pregnancy, however, there are other reasons for failure to return to estrus including pseudopregnancy in goats or variability in estrous cycle length at the end of the breeding season. Waiting for lambing or kidding may be perceived by some producers as a method that can be implemented to save on the costs of pregnancy diagnosis but ends up being more costly when a doe or ewe that has incurred feed costs to the farm fails to deliver lambs or kids at the end of the expected gestation. The 2 most reliable methods for determination of pregnancy status in small ruminants, which will be the focus of this review, are ultrasonography and detection of pregnancy-associated glycoproteins (PAG) via blood-based pregnancy tests. Whichever method is chosen, it should be economical, highly accurate, easily applicable in field conditions, and enable the practitioner to diagnose pregnancy early in gestation.

Ultrasonography

Ultrasonography is a common method of pregnancy diagnosis in sheep and goats and may be performed either transabdominally or transrectally using a variety of commercially available, portable ultrasound units. Units equipped with a linear, variable frequency transducer ranging from 5-7.5 MHz, which are commonly used in bovine reproductive herd management, can also be used for small ruminant pregnancy diagnosis. When performing transrectal ultrasound in small ruminant species, a probe extender may be used but the operator should ensure that the animals are well restrained and the procedure is performed with caution to avoid trauma to the rectal mucosa.

In addition to confirmation of pregnancy status, the use of ultrasound provides additional benefits to the clinician. Ultrasound allows for more accurate estimation of gestational days which enables the producer to group ewes or does by expected date of parturition. Furthermore, determination of fetal number aids in nutritional management of ewes or does carrying multiple fetuses for prevention of pregnancy toxemia in the periparturient period. Determination of fetal sex using ultrasound may also be valuable for inventory management of the herd or flock. Finally, ultrasound allows for the determination of fetal viability and detection of any uterine pathology that may be detrimental to the reproductive fitness of the ewe or doe.

In small ruminants, anechoic fluid in the uterine lumen may be detected via as early as 17-19 days gestation via transrectal ultrasonography or 25-28 days transabdominally. Fluid within the uterine lumen should not be the sole basis of pregnancy confirmation and should be accompanied by identification of the amniotic vesicle and a fetal heartbeat for definitive diagnosis. Using a transrectal approach, a fetal heartbeat has been detected as early as 16 days gestation in the ewe and 22-23 days gestation in the doe. When scanning via a transabdominal approach, a fetal heartbeat can be reliably detected in both ewes and does by 27-30 days gestation.[1]

As pregnancy progresses and the uterus descends into the abdominal cavity, the transabdominal approach for evaluation of pregnancy is preferred. The proximity of the uterus relative to the abdominal wall enables visualization of fetal anatomy and measurements for determination of gestational age (Table 1). The most reliable measure for determining gestational age is the crown rump length (CRL) of the fetus. This measure is only possible prior to 40 days gestation in the sheep and 50 days gestation in the goat as the fetus enlarges to the point that it extends beyond the limits of the ultrasound screen, although these limits to CRL measurement may vary depending on breed and number of fetuses.[1] Beyond 40-50 days gestation, other measures may be used to estimate fetal age including biparietal diameter, thoracic diameter and head length.

Table 1: Approximate fetal measurements, in centimeters, for estimation of gestational age in sheep and goats by ultrasound.[2]

	24-35 days	36-40 days	41-65 days	66-130 days
Embryonic vesicle (cm)	2.0-3.5	3.5-5.0	>5.0	
Crown rump length (cm)	1.0-2.0	2.0-3.5	>3.5	
Thoracic diameter (cm)	0.5-1.2	1.2-1.5	1.5-3.0	3.0-8.0
Biparietal diameter (cm)			1.2-2.5	2.5-5.0
Head length (cm)			1.5-3.0	3.0-7.5

Pregnancy-associated glycoprotein assays

Blood-based pregnancy tests for the detection of pregnancy-associated glycoproteins (PAGs) in maternal serum have become more common in recent years. The commercially available PAG tests are based on bovine PAG antibodies, however, due to similarities in the structure of all ruminant PAGs, there is cross-reactivity in the assays between bovine and sheep and goat PAGs. This allows for the use of commercially available bovine tests to detect pregnancy in small ruminants and other ruminant species. There are over 10 closely related PAG proteins in ruminants,[3] including pregnancy specific protein B (PSPB) and pregnancy-associated glycoprotein-1 (PAG-1) which form the basis for the BioPRYN® and IDEXX assays, respectively.

Pregnancy-associated glycoproteins are inactive aspartic proteinases secreted by the trophoblast layer of the ruminant placenta and enter maternal circulation around the time of placental attachment.[4] There are many factors that may affect maternal concentrations of PAG throughout gestation including breed or subspecies, fetal sex or number, birth weight, maternal nutritional status, environmental conditions, and the type of assay used for detection.[4] While PAG assays may be useful when ultrasound diagnosis of pregnancy is not available, there are limitations to the use of PAG as compared to ultrasound including the persistence of PAG in maternal serum for a variable amount of time following fetal loss leading to a false positive diagnosis, and the inability to determine fetal viability or visualize uterine pathology.

Pregnancy-associated glycoproteins have also been used as a marker of the viability of the feto-placental unit. Declines in PAG have been associated with embryonic or fetal loss in cattle and goats.[5,6] Experimental inoculation with abortifacient organisms *Toxoplasma gondii and Listeria monocytogenes* resulted in decreases in PAG levels in maternal serum.[6] Lower PAG in heat stressed cattle during late gestation indicates that the effects of heat stress extend to the level of the feto-placental unit.[7] Furthermore, significant decreases in maternal PAG were identified in a sheep model investigating the effects of gestational exposure to the endocrine disrupting chemical, bisphenol S.[8] Although the exact function of PAG is unknown, they serve as a useful marker for detection of pregnancy as well as an indicator of placental dysfunction during gestation.

Pregnancy-associated glycoprotein levels in maternal serum can vary depending on the assay used. When detecting PSPB in sheep using the BioPRYN assay, levels detected exhibit a bimodal pattern reaching peaks around 50 and 120 days gestation with a nadir around 90 days gestation.[9] However, when using the IDEXX Bovine Pregnancy Test for detection of PAG-1 in sheep, levels steadily increase from early gestation through parturition.[4] From a clinical perspective, it is important to recognize these differences in assay patterns as sampling at the gestational timepoints during the nadir of detectable PAG could result in a false negative result. When possible, ultrasound should be used on any inconclusive results from blood-based pregnancy tests.

Most blood-based pregnancy tests require samples to be sent to a reference laboratory for analysis. However, the IDEXX Rapid Visual Pregnancy Test enables pregnancy diagnosis via visual observation of a color change in the test well following a short incubation time and without the need for expensive equipment to determine optical density. This bench-top assay, based on the same bovine PAG antibody as the IDEXX Bovine Pregnancy Test, allows practitioners to provide in-house pregnancy diagnosis for bovine and small ruminant clients.

In a study comparing the results of the IDEXX Rapid Visual Pregnancy Test to ultrasound and lambing records, sensitivity and specificity were 97.3% and 94.3%, respectively, in ewes sampled at 35-92 days gestation.[10] Results of this study in sheep were comparable to an evaluation of the assay in cattle where sensitivity and specificity were 98% and 85%, respectively.[11] In the cattle study, the lower specificity was attributed to errors in the visual readout as the specificity improved to 94% when a spectrophotometer was used to determine optical density of the sample.[11] When evaluated in goats, the sensitivity and specificity of the IDEXX Rapid Visual Test were 94.1% and 80.5%, respectively, with the lower specificity being attributed to errors in visual readout or timing of the blood sample collection relative to ultrasound diagnosis. Regardless of which assay is used to detect pregnancy in small ruminants, sensitivity and specificity for these tests are highest after 30 days gestation.[12]

Determination of fetal number

Determination of fetal number in small ruminants can be an important aspect of pregnancy diagnosis. Knowledge of fetal number allows producers to adjust their nutritional management to prevent pregnancy toxemia in these litter-bearing species. The gold standard for determination of fetal number is examination via ultrasound. Fetal counts can be determined with greater than 80% accuracy if performed between approximately 30-70 days gestation.[1] After 70 days gestation, it can be difficult to distinguish between multiples due to increasing fetal mass. A good rule of thumb to maintain accuracy in diagnosing multiple fetuses is to only count when multiple fetuses can be visualized on the same field of view with the ultrasound. Ultimately, determination of fetal number can increase the

amount of time required for examination of the ewe or doe and the value of this information is dependent on how the client will use the information provided by the veterinarian. If no management changes will be implemented based on fetal number, it may not be worthwhile to spend the extra time on counting fetuses during pregnancy diagnosis.

Some studies have evaluated the utility of optical density readouts of PAG ELISA assays for determination of fetal number in sheep and goats. While PAG values are higher in twin pregnancies in sheep compared to singletons, a limitation to the use of PAG assays for fetal number is that it is not possible to determine fetal number beyond twins.[4,14] In goats, PAG levels for does carrying twins is significantly greater than those carrying singletons from 28-93 days gestation.[15] Despite studies indicating the ability to detect multiples using a PAG assay, it is important to note that there is a high level of variability in PAG levels between and within animals and serial sampling would likely be required to accurately determine fetal number using a blood-based test.

Conclusion

Pregnancy diagnosis in small ruminant flocks and herds is key to maintaining profitability for the producer. Determination of pregnancy status, gestational age, and fetal number gives veterinarians an opportunity to aid clients in providing a high level of care and nutritional management during gestation and through the periparturient period. The availability of ultrasound and blood-based pregnancy tests and ease of implementation allows flexibility for both the veterinarian and producer in attaining their herd or flock production goals.

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