

ACCURACY OF TRANSVAGINAL ULTRASONOGRAPHY FOR EARLY PREGNANCY DIAGNOSIS IN ATTAPPADY BLACK GOATS

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ABSTRACT. Transabdominal ultrasonography has been popularly used in animal reproduction for the assessment of pregnancy status and foetal viability where as transvaginal method for pregnancy diagnosis is rarely used for pregnancy diagnosis in goats. The study was conducted in 74 Attappady Black goats from Government Goat Farm, Attappady and Livestock Research Station, Thiruvizhamkunnu to evaluate the accuracy of trans-vaginal methods and to identify the fetal characteristics from day 20 to 75. Transvaginal ultrasonographic examination was performed using endocavity transducer with frequency of 6.5 to 8 MHz. Observations were made in all the pregnant does at three different stages of pregnancy i.e., 20-35 days, 35-55 days and 55-75 days. Does were diagnosed as pregnant from the observation of gestational sac, embryo or foetus, embryonic or foetal heartbeat, placentomes or foetal skeleton. Sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy along gestation period were 98.03, 92.3, 96.15, 96 and 95.62%, respectively. It is concluded from the present study that transvaginal ultrasonography can be used for herd pregnancy diagnosis at early stages from day 20 to 45 of pregnancy diagnosis in goats.

INTRODUCTION

Abdominal palpation and udder enlargement have been used traditionally for pregnancy diagnosis in goats but applicable only in later stages of pregnancy. A reliable method for early pregnancy diagnosis would allow early culling or rebreeding of non-pregnant does. One of the most important reasons for pregnancy diagnosis is detection of pseudopregnancy or hydrometra. Modern diagnostic methods like radiography, measurement of plasma progesterone concentration, assessment of estrone sulfate, evaluation of pregnancy specific proteins, and ultrasonography have been used to diagnose pregnancy in goats. B-mode transrectal and transabdominal ultrasonography provided accurate early pregnancy diagnosis and foetometry in goats (Ameer, 2008). Transabdominal scanning has been shown to be effective and reliable under field conditions for pregnancy diagnosis in goats (Hesselink and Taverne, 1994). Recent investigations have revealed that transrectally performed ultrasonography is easier to diagnose early pregnancy in goats (Gordon, 2004). However, faecal pellets and rectal brittleness are potential drawbacks of transrectal ultrasonography (Aria *et al.*, 2004). There is also increased risk of infection and luminal wall injury or bleeding during transrectal

examination. Also the sensitivity of the transrectal scans may decrease in the absence of overnight fasting (Karen *et al.*, 2009). Despite routine use in humans, transvaginal method for pregnancy diagnosis is rarely used in goats. This study was conducted to identify the early stage foetal structures which could be detected by transvaginal ultrasonography, and to evaluate the accuracy of transvaginal methods in detecting pregnancy and foetal structures in first 75 days of pregnancy in Attappady goats.

MATERIALS AND METHODS

The study was conducted in 74 Attappady Black goats from Government Goat Farm, Attappady and Livestock Research Station, Thiruvizhamkunnu. Ultrasound scanning was conducted in all the animals from day 20 after natural service to day 75 using a real-time scanner (Mindray, China) to identify the early stage gestational structures. Transvaginal ultrasonographic examination was performed using endocavity transducer with frequency of 6.5 to 8 MHz. The does were restrained in standing position against wall by an assistant. The probe was lubricated with ultrasound gel, protected with a disposable sheath. The probe was gently introduced into the vagina first at a 45° of upward angle, then forward and straight cranially. The entire pelvis was visualized with 90° rotations and preferred frequency was 7.5 MHz. If visualization was not proper, the abdominal wall of the animal was then lifted gently with the right hand. The probe was protected with a disposable sheath and ultrasound gel was

applied to the transducer to assure good acoustic transmission. Observations were made in all the does at 3 different stages of pregnancy i.e., 20-35 days, 35-55 days and 55-75 days. Does were diagnosed pregnant from the observation of gestational sac, embryo or foetus, embryonic or foetal heartbeat, placentomes or foetal skeleton. Sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy of transvaginal ultrasonography was calculated (Raja-Khalif *et al.*, 2014). The accuracy in diagnosing pregnancy status by the transvaginal ultrasound scanning technique was compared using the actual kidding data.

RESULTS

Out of seventy-four does tested for pregnancy, 52 does were diagnosed as pregnant by day 20 to 75 of gestation. Table 1 shows the percentage of ultrasonographic observations of foetus and gestational structures obtained by transvaginal ultrasound scanning at 3 different stages. During day 20 to 35, embryonic vesicle, embryo and foetal heart beat were clearly visible with transvaginal probe. The embryonic vesicle, which appeared as a sharply demarcated anechoic, round or oval structure was observed earliest on day 20 of gestation. The embryo appeared as an echogenic structure within the anechoic sac (Figure 1) on day 22 and foetal heart was first detected as a flickering. From day 22 onwards embryo could be visible within the gestational sac by transvaginal scanning (Figures 2 to 4). Placentomes were first observed as a concave shaped echogenic

Table 1. Performance of transvaginal ultrasonography for diagnosing early foetal structures

Foetal Development characteristics	Observations of transvaginal method					
	20-35 days	%	35-55 days	%	55-75 days	%
Gestational Sac	42/52	80.76	36/52	69.23	-	-
Embryo within the Sac	44/52	84.61	38/52	73.07	-	-
Placentomes	0/20	-	39/52	75	52/52	100
Skull and Leg Buds	-	-	26/52	41.93	6/52	11.53
Ribs and Vertebrae	-	-	18/52	34.61	11/52	21.15
Fetal Heart	36/52	69.23	16/52	30.76	-	-
Foetal abdomen	-	-	-	-	-	-
Fetal Number	-	-	-	-	-	-
Fetal Viability	36/52	69.23	46/52	88.46	-	-

Table 2. Efficacy of transvaginal ultrasonography for early pregnancy diagnosis

Sl. no	Diagnosis	Transvaginal Ultrasonography
a	Correct positive	50/52 96.15
b	Incorrect positive	2/52 3.84
c	Correct negative	24/25 96.0
d	Incorrect negative	1/25 4.0
e	Sensitivity (%)	50/50+1 98.03
f	Specificity (%)	24/2+24 92.3
g	Positive predictive value (%)	50/52 96.15
h	Negative predictive value (%)	24/25 96
i	Overall accuracy (%)	95.62

- a: Pregnant does that were correctly diagnosed;
 b: Pregnant does that were incorrectly diagnosed;
 c: Non-pregnant does that were correctly diagnosed;
 d: Non-pregnant does that were incorrectly diagnosed;
 e: Sensitivity $[a/(a+d)]$;
 f: Specificity $[c/(b+c)]$;
 g: Positive predictive value $[a/(a+b)]$;
 h: Negative predictive value $[c/(c+d)]$;
 i: Overall accuracy $[(e+f+g+h)/4]$



Figure 1. Embryo as an echogenic structure within the anechoic sac on day 22



Figure 2. Embryo within the gestational sac on day 24



Figure 3. Pregnant uterus with Embryo on day 28



Figure 4. Embryo on day 32 on transvaginal ultrasonography



Figure 5. Placentomes as a concave echogenic structure on day 42 of gestation



Figure 6. Foetal skeleton on transvaginal ultrasonography

structure on day 42 of gestation (Fig. 5) and foetal skeleton was observed between day 45 to 50 gestation (Fig. 6) by employing transvaginal scanning. Foetal viability was assessed based on the observation of foetal heart beat.

Pregnant does that were correctly diagnosed were given as true positive and were incorrectly diagnosed as false positive. Non-pregnant does that were correctly diagnosed as true negative and non-pregnant does that were incorrectly diagnosed as false negative. The values of true positive, false positive, true negative and as false negative were 96.15, 3.84, 96.0 and 4.0 respectively. Sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy along gestation period were 98.03%, 92.3%, 96.15%, 96.00% and 95.62%, respectively. Accuracy of pregnancy diagnosis was confirmed by kidding.

DISCUSSION

Attappady Black goats, reared mainly by the tribes of the Attappady region, the hilly terrains of Palakkad district of Kerala for meat purposes and have evolved over a long period adapting to the agro-ecological conditions of the area and are resistant to many diseases. The total number of this breed of goats is less than 6000 and due to indiscriminate breeding with other types of bucks, the purity of this group is becoming increasingly diluted. Therefore there is an urgent need for the conservation and genetic improvement of this unique goat genetic resource of India (Stephen *et al.*, 2005). The development of a method to

accurately estimate the stage of pregnancy when precise mating dates are not available would assist management to maximize survival rates of offspring (Greenwood *et al.*, 2002). Real-time ultrasound evaluation of small ruminants offers an unparalleled range of information regarding pregnancy status, number of fetuses and gestational age (Haibel G.K., 1990). Diagnosing pregnancy and determining fetal number are important tools for organising appropriate nutritional management of ewes, mating non-pregnant does in the same breeding season, reducing the incidence of dystocia, determining fertility problems, and preventing the slaughter of pregnant females. Traditional methods for pregnancy diagnosis in small ruminants are palpation through external abdomen and noting udder enlargement. However, these methods are applicable only in late pregnancy.

The technique of transabdominal ultrasonography has been used with great accuracy as a means for pregnancy diagnosis and estimation of fetal numbers in goat (Abdelghafar *et al.*, 2011) and sheep (Anwar *et al.*, 2008). Transabdominal ultrasonography has been popularly used in animal reproduction for the assessment of pregnancy status and foetal viability. Studies regarding Transvaginal ultrasonography in goats for early pregnancy detection are limited. So the present study was conducted to to evaluate the accuracy of transvaginal methods in detecting pregnancy and foetal structures in first 75 days of pregnancy in Attappady goats so as to develop an easier technique of pregnancy diagnosis in goats. Trans-vaginal approach has long been neglected in Veterinary Medicine, while it has

been largely adopted in human obstetrics and gynaecology. On experiences on transvaginal pregnancy diagnosis in cattle, Aria *et al.* (2005) suggested its application in adult small ruminants, in which our probe could be inserted in the vagina, excluding the nulliparous ewe-lambs, characterised by a very thin vaginal lumen. Aria *et al.* (2004) evaluated the on-field practicability of transvaginal diagnosis in bovines and concluded that transvaginal ultra-sound scanning could represent easy and alternative method to trans-abdominal scanning. Foetus and foetal structures could be identified in pregnant goats using transvaginal probe at 3 different stages of pregnancy i.e., 20-35 days, 35-55 days and 55-75 days. Ultrasonographic images obtained in transvaginal study were similar with those reports by Gonzalez *et al.* (2004) by transrectal ultrasonography. Martinez *et al.* (1998) detected anechoic area representing amniotic fluid that facilitated diagnosis of pregnant does as early as day 18 of gestation using transrectal probes. Padilla *et al.* (2005) proposed that the best time to distinguish between single and twin foetuses in goats with a 7.5 MHz rectal probe was between 28 and 40 days of gestation and difficulty in identifying the foetal number increased with multiple pregnancies.

Foetal heart and embryo were observed on days 21 (Martinez *et al.*, 1998), 23 (Padilla-Rivas *et al.*, 2005) and 24 of pregnancy (Medan *et al.*, 2004). In this study, foetal heart was detected on day 28 of gestation. Detection of foetal heart was suggested as a significant indicator for pregnancy diagnosis (Martinez *et al.*, 1998; Medan *et al.*, 2004). After 50 days, spinal

cord, ribs and limbs could be partially visible upon lifting the abdomen with the help of an assistant. Foetal characteristics could be rarely detected ultrasonographically with transvaginal probe during day 55 to 80. In the present study sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy along gestation period were 98.03%, 92.3%, 96.15%, 96.00% and 95.62%, respectively were similar to the studies of Koker, *et al.* (2012). Raja-Khalif *et al.* (2014) compared effectiveness of transrectal and trans-abdominal probes and observed similar results in terms of accuracy as obtained in the present study.

CONCLUSION

Transvaginal ultrasonography can be used for early pregnancy diagnosis in goats and it can be performed with less patient preparation, less time consuming, with less risk of luminal wall injury and bleeding. The first stage of gestation gave slightly a lower percentage of positive diagnosis using transabdominal probe that could be due to the difficulty in identifying structures from days 20 to 35 of gestation. From second stage of gestation onwards, transvaginal approach is not practical due to the difficulties in obtaining images. Distress, bleeding or interruptions of pregnancies were not seen after transvaginal ultrasonographic examinations. Thus, it can be concluded from this study that transvaginal ultrasonography could represent a safe, easy and alternative method to trans-abdominal scanning in farm conditions for herd pregnancy diagnosis.

REFERENCES

1. Abdelghafar R.M., Ahmed B.H., Ibrahim M.T. and Mantis P. (2011). Prediction of gestational age by transabdominal real-time ultrasonographic measurements in Saanen goats (*Capra hircus*). *Global Veterinaria* **6**:346-351
2. Amer H.A. (2008). Determination of first pregnancy and foetal measurements in Egyptian Baladi goats. *Vet. Ital.* **44**:429-437.
3. Anwar M., Riaz A., Ullah N. and Rafiq M. (2008). Use of ultrasonography for pregnancy diagnosis in Balakhi sheep. *Pakistan Vet. J.* **28**(3):144-146.
4. Aria G., Shau Z., Botta R., Giuliotti L. and Rota A. (2004). Transvaginal echographic approach to early pregnancy diagnosis in small ruminants. *Ann. Fac. Med. Vet. Pisa* **57**:99-105.
5. Aria G., Botta R., Sbrana A. and Panzani D. (2005). Accertamento di gravidanza per via ecografica transvaginale nella specie bovina. *Large An. Rev.*, **2**:25-31.
6. Gonzalez F.C., Batista M., Rodriguez N., Alamo D., Sulon J., Beckers J.F. and Gracia A. (2004). A comparison of diagnosis of pregnancy in the goat via transrectal ultrasound scanning, progesterone and pregnancy-associated glycoprotein assays. *Theriogenology*, **62**: 1108-1115.
7. Gordon I. (2004). *Controlled Reproduction in Sheep and Goats*, third ed. CABI Publishing, Oxon.
8. Greenwood P., Slepetic R., Mcphee M. and Bell A. (2002). Prediction of stage of pregnancy in prolific sheep using ultrasound measurement *J. Reprod. Fertil. Dev.*, **14**:7-13.
9. Haibel G.K., (1990). Use of ultrasonography in reproductive management of sheep and goat herds. *Veterinary Clinics of North America Food Animal Practice*, **6**: 597-613
10. Hesselink J.W., Taverne M.A. (1994). Ultrasonography of the uterus of the goat. *Vet. Q.* **16**: 41-45.
11. Karen M.A., Sayed E., Fattouha M., Saber S.A.Z. (2009). Estimation of gestational age in Egyptian native goats by ultrasonographic fetometry. *Anim. Reprod. Sci.* **114**: 167-174.
12. Koker A., Ince D. and Sezik M. (2012). The accuracy of transvaginal ultrasonography for early pregnancy diagnosis in Saanen goats: A pilot study. *Small Rumin. Res.* **105**: 277-281
13. Martinez M.F., Bosch P., Bosch R.A. (1998). Determination of early pregnancy and embryonic growth in goats by transrectal ultrasound scanning. *Theriogenology* **49**: 1555-1565.
14. Medan M., Wananabe G., Absy G., Sasaki K., Sharawy S. and Taya K. (2004). Early pregnancy diagnosis by means of uotasonography as a method of improving reproductive efficiency in goats. *J. Reprod. Dev.* **50**: 391-397.
15. Padilla-Rivas G.R., Sehnrey B. and Holtz W. (2005). Early pregnancy detection by real-time ultrasonography in Boer goats. *Small Rumin. Res.* **58**: 87-92.
16. Raja-Khalif R.I.A., Rahman M.M., Wan-Khadijah W.E. and Abdullah R.B. (2014). Pregnancy diagnosis in goats by using two different ultrasound probes *J. Anim. Plant Sci.* **24**: 1026- 1031.
17. Stephen M., Raja T.V. and Sosamma I. (2005). Survey and characterisation of Attappady Black goats of Kerala, India. In: *Animal Genetic Resources Information*, **37**:43-51.

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