Small Ruminant Research 85 (2009) 135–141

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Evaluation of different techniques for pregnancy diagnosis in sheep

B.A. Ganaie[a], M.Z. Khan[a], R. Islam[a,*], D.M. Makhdoomi[b], S. Qureshi[c], G.M. Wani[a]

[a] Division of Animal Reproduction, Gynaecology and Obstetrics Faculty of Veterinary Sciences and Animal Husbandry, S. K. University of Agricultural Sciences and Technology (K), Shuhama, Alusteng, Srinagar-190006, Kashmir, India
[b] Division of Veterinary Surgery and Radiology, SKUAST-K, Srinagar-190006, India
[c] Division of Veterinary Microbiology and Immunology, SKUAST-K, Srinagar-190006, India

ARTICLE INFO

Article history:
Received 18 October 2008
Received in revised form 21 August 2009
Accepted 2 September 2009
Available online 26 September 2009

Keywords:
Pregnancy diagnosis
Sheep
Ultrasonography
Preg-alert
Doppler ultrasonic equipment
Progesterone assay

ABSTRACT

Fifty Corriedale ewes were used in this study to evaluate pregnancy diagnosis in sheep. Ewes were bred under a pen mating system and pregnancy diagnosis was initiated from day 15 post-mating, applying the diagnostic techniques of trans-abdominal real-time B-mode ultrasonography, Preg-alert (A-mode ultrasonography), the Doppler ultrasonic fetal pulse detector or the plasma progesterone concentration assay (EIA). These tests were repeated fortnightly on all the ewes until the onset of lambing. The accuracy of trans-abdominal real-time B-mode ultrasonography (68%) at days 15–30 of pregnancy increased to 100% by days 61–75 and remained constant until lambing. The accuracy of the Preg-alert (56%) diagnosis at days 31–45 increased to 94% by days 91–105 of gestation and then decreased to 82% from days 136 of gestation to lambing. The accuracy of both the Doppler ultrasound (56%) at days 31–45 and plasma progesterone assay (98%) at days 15–30 of gestation increased to 100% at days 76–90 and 46–60 of gestation, respectively and remained constant until parturition. The mean plasma progesterone concentration at days 0–6 (1.41 ± 0.21 ng/ml) increased to 4.0 ± 0.87 ng/ml at days 16–30 (days 18.23 ± 0.78) post-mating. Animals returning to estrus recorded less than 1 ng/ml at days 18.23 ± 0.78 post-mating. The accuracy of both the B-mode ultrasonic technique (78%) and plasma progesterone assay (98%) was significantly higher ($P < 0.05$) than the accuracy obtained with the A-mode and Doppler ultrasound (both 56%) at days 31–45 of gestation. The study concluded that real-time B-mode ultrasonography is the earliest, most accurate, safest, fastest and most economical method of pregnancy diagnosis in sheep at farm level. The A-mode and Doppler methods can also be used under field conditions, where sophisticated laboratory facilities are not available. Plasma progesterone assays (EIA) can be used as a means of early pregnancy diagnosis in organized sheep farms with fair accuracy.

1. Introduction

Diagnosing pregnancy at an early stage is of great economic importance in sheep production, because of the seasonal breeding patterns of sheep—where loss of a breeding opportunity means loss of one productive year for the ewe. Intensive sheep management and the wide spread use of controlled breeding techniques together with AI and out-of-season breeding has increased the need for an accurate and practical tests for early pregnancy diagnosis. Reproductive and production losses in the form of abortions, stillbirths and the birth of weak lambs can be reduced by separating the ewe flock into pregnant and non-pregnant groups (Wani et al., 1998). In addition, accurate information of the stage of gestation will be useful to dry off lactating females at a convenient stage and to monitor females near to term (Doize et al., 1997). Reliable techniques for early pregnancy diagnosis then also help in the

* Corresponding author. Tel.: +91 194 2262208; fax: +91 194 2262207.
E-mail address: rafiqvet@gmail.com (R. Islam).

0921-4488/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.smallrumres.2009.09.003

culling or rebreeding of barren ewes and does and thus provide a valuable tool to be used in controlled breeding programmes.

Various methods have been used to diagnose pregnancy in sheep. "Less practical methods" include the management method (non-return to estrus), abdominal palpation, laparotomy, peritonoscopy and the rosette-inhibition test (Ishwar, 1995). "More practical methods" include radiography, pregnancy protein assays, hormonal determination and ultrasonography. Palpation of the genital tract per rectum facilitates the early pregnancy diagnosis in large domestic animals like cows and mares, which is not anatomically possible in sheep and goats. The visual assessment via abdominal palpation does give an indication of pregnancy, but with low accuracy and only at an advanced stage, when it is of less economic importance. In addition laparotomy, laparoscopy, the rosette-inhibition test and vaginal biopsies are accurate techniques—however, these methods are impractical under farm conditions (Goel and Agrawal, 1992; Gordon, 1999). Among the practical methods of pregnancy diagnosis, the estimation of blood progesterone levels of ewes at day 18 post-breeding has recorded reliable accuracy for pregnancy diagnosis. Presently three types of ultrasonic systems are being used for pregnancy diagnosis in small ruminants, viz. A-mode (amplitude-depth), real-time B-mode (brightness mode) and the Doppler (D-mode) ultrasound. The ultrasonic method is free of any negative effects on the fetus, or the dam-unlike radiography and laparoscopy and has gained wide acceptance in recent years. The present study was performed to compare the three ultrasonic techniques and the plasma progesterone assay (EIA) in terms of accuracy for pregnancy diagnosis in Corriedale ewes at various stages of gestation.

2. Materials and methods

2.1. Experimental ewes and management

The study was carried out at Sheep Research Station of Sher-e Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Alusteng, Srinagar, at a latitude of 34°5′ North and longitude of 74°48′ East in the centre of the Kashmir valley (Lawrence, 2000). Fifty Corriedale ewes that had previously lambed were selected for this study. The weights of the ewes at the time of breeding were 35–40 kg, while the age ranged between 3.5 and 4.5 years. All ewes were maintained under uniform management conditions and reared under a semi-intensive housing system during September to October (autumn), while from November to March (winter) animals were reared under an intensive housing system, due to the low ambient temperatures (−7 to 15 °C).

2.2. Estrus detection and mating

Estrus was detected in all ewes with the aid of a Draminski Estrus Detector (Draminski Electronic in Agriculture, PL-10-860 Olsztyn, Owocowa 17). The occurrence of estrus was detected by the measuring of the vaginal electrical resistance (Intan-Shameha et al., 2005). Estrus was also reconfirmed by the observation of the mating paint marks, made by the rams, on the rump of the ewes. All experimental ewes were bred using a pen mating system during the autumn breeding season (September to November). The ewes detected in estrus were kept in tupping pens, using healthy, fertile breeding rams. The brisket region of the rams was smeared with greasy paint and ewes were considered to be mated when colour marks were found on their rumps. The day of mating was recorded as day 0, for calculating the approximate gestational stage of the ewe—which was also later confirmed from the lambing data.

2.3. Pregnancy diagnosis

Pregnancy diagnosis was initiated from day 15 post-mating, using the respective diagnostic methods and this was repeated fortnightly until the onset of lambing. The various methods of pregnancy diagnosis used on the ewes can be described as follows.

2.3.1. Trans-abdominal real-time B-mode ultrasonography

All the ewes were scanned trans-abdominally, using a real-time ultrasound scanner equipped with a 3.5 MHz sector array transducer (Sonalisa-32 ultrasound scanner, Larsen and Toubro Limited). The lower abdominal region of the ewes was prepared for scanning by shaving the wool and ewes restrained in both the dorsal, as well as the lateral recumbencies by two attendants. An ultrasonic jelly was applied on the shaven area to be scanned before attaching the probe. Pregnancy was confirmed by detection of an enlarged uterine lumen, with a dark spherical amniotic cavity and the presence of an embryo, circular, semi-circular and cup shaped placentomes, fetal heart beats, fetal movements and the presence of the fetal skeleton. During the scanning procedure the visibility of urinary bladder served as a landmark (Padilla-Rivas et al., 2005; Suguna et al., 2008).

2.3.2. Preg-alert (A-mode) ultrasonography

All ewes were scanned trans-abdominally using a "Renco-Preg-alert" (RENCO corporation© 1995 U.S. PAT 4112927, USA), an A-mode ultrasonic device with a trans-abdominal probe. The ewes were restrained by an attendant in two positions, viz. "made to sit on their tails" (sitting procedure), and standing position (standing procedure) and scanned in both positions. Before scanning, the wool free area just lateral to the right mammary gland was cleaned and then a conductive fluid (cooking oil) was applied to the desired scanning site. The function knob was set to the S-mode (Sheep-Pregnancy mode), the Sens control was set at the 12 o'clock position and the tone switch turned on. The probe was placed on the skin and directed across the abdomen towards the left last rib (standing procedure). Precautions were taken not to direct the probe towards the tail, in order to prevent a false pregnancy signal from the bladder. In the case of the sitting procedure, the probe was aimed towards the hip bones on either side of the uterus. The ewes were scanned at the rate of one ewe per minute in both the sitting and standing procedures.

Pregnancy was indicated by a spike in the last third of the second zone or anywhere in the third or fourth zones of the oscilloscope screen. The tone was also taken as an indicator of pregnancy. Sounds were only heard when the spike enters the third/fourth zone of the screen. No spike in the specific zones and no tones indicated a negative diagnosis for pregnancy (Anonymous, 1995).

2.3.3. Doppler ultrasonic equipment

All ewes were subjected to pregnancy diagnosis using a Medata type of Doppler ultrasonic apparatus, provided with a rectal probe. The ewes were restrained in standing position and ultrasonic jelly was used to lubricate the probe before inserting it into the rectum. The probe was gently moved inside with its plain surface directed ventrally. Pregnancy was diagnosed on the basis of three characteristics recorded, namely middle uterine artery sounds (MUAS), umbilical sounds (UMS) and fetal heart beats (FHB). These sounds were monitored through a head phone. The MUAS was registered as a "gushing sound", the UMS as "swishing sound" while as the FHB as a "galloping sound" (Wani et al., 1998).

2.3.4. Plasma progesterone assay

2.3.4.1. Blood collection. Five millilitres of jugular blood was collected from ewes at days 0, 7, 18, 30, 45, 60, 75, 90, 105, 120, 135 and 150 post-mating and 7 and 15 days post-lambing into heparinised test-tubes and transported to the laboratory in an ice box. After collection, the blood was centrifuged at 3000 rpm for 15 min and the plasma aspirated and kept at −20 °C until assayed for progesterone concentration.

2.3.4.2. Plasma progesterone concentration determination. The plasma progesterone concentration was determined using the enzyme immunoassay (EIA) technique (Boscos et al., 2003), using progesterone ELISA kits (EIAgen PROGESTERONE, Ref LI.4010.K, Adaltis Italia S.P.A., Italy).