ELSEVIER

Comparison of accuracy of transabdominal ultrasonography, progesterone and pregnancy-associated glycoproteins tests for discrimination between single and multiple pregnancy in sheep

Aly Karen [a,*], Bouchra El Amiri [b], Jean-Francios Beckers [b],
Jose Sulon [b], Marcel A.M. Taverne [c], Ottó Szenci [d]

[a] *Department of Theriogenology, Faculty of Veterinary Medicine, Tanta University, Kafr El-Sheikh, P.O. Box 33516, Egypt*
[b] *Department of Physiology of Reproduction, Faculty of Veterinary Medicine, Bd de Colonster
No. 20, B41, B-4000 Sart Tilman, Belgium*
[c] *Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, The Netherlands*
[d] *Clinic for Large Animals, Faculty of Veterinary Science, Szent István University,
H-2225 Üllő-Dóra Major, Hungary*

Abstract

The aim of the present study was to evaluate and, compare the accuracy of transabdominal ultrasonographic (US) and the progesterone (P4-RIA) and ovine pregnancy-associated glycoprotein (ovPAG-RIA) tests for the discrimination between single and multiple pregnancy in sheep.

One hundred pregnant Awassi × Merino ewes were scanned by transabdominal ultrasonography (3.5 MHz linear-array transducer) at Days 43–56 and 81 of these ewes were scanned at Days 76–87 of gestation. The ewes were scanned in dorsal recumbency at the bare area of the inguinal regions (without pre-scanning shaving of the ventral abdominal wall). After each scan, blood samples were withdrawn from the jugular vein to estimate the levels of P4 and ovPAG by radioimmunoassay.

At lambing, 61 ewes gave birth to single lambs and 39 ewes gave birth to multiples. The sensitivity of the transabdominal US, the P4-RIA and the ovPAG-RIA tests for determining ewes carrying multiples was 54, 64.1 and 64.1% at Days 43–56. At Days 76–87 of gestation these accuracies were 60.0, 66.7 and 76.6% for the US, P4-RIA and PAG-RIA tests, respectively. The specificity of the transabdominal US, the P4-RIA and the ovPAG-RIA tests for determining ewes carrying singles, was 78.6, 60.7 and 62.3% at Days 43–56 and 78.4, 64.7 and 70.6% at Days 76–87 of gestation, respectively. It is concluded that the accuracy of transabdominal ultrasonographic (without pre-scanning shaving of the ventral abdominal wall), the P4- and the ovPAG-RIA tests for determination of the fetal numbers in Awassi × Merino crossbred ewes is too low to be used in the field.
© 2005 Elsevier Inc. All rights reserved.

Keywords: Transabdominal ultrasonography; Progesterone; Ovine pregnancy-associated glycoprotein; Sheep; Litter size

1. Introduction

Determination of fetal numbers is desirable for better management of pregnant sheep. It offers producers the opportunity to feed the groups of ewes according to

---

* Corresponding author. Tel.: +20 48 2910343;
fax: +20 47 3231311.
E-mail address: alykaren@hotmail.com (A. Karen).

their precise needs in late gestation, which will prevent pregnancy toxemia, optimize the birth weights and increase the survivability of lambs born to ewes bearing multiples. In addition it decreases the incidence of dystocia and the cost of feeding in ewes carrying singletons [1].

Several methods have been attempted to determine the number of fetuses in sheep and have been reviewed recently [2]. Recto-abdominal technique was of low accuracy for determination of multiples (57%) and hazardous with respect to rectal injury and abortion [3,4]. Radiography is an accurate method for determination of fetal numbers after Day 70, however the cost of the portable X-ray, the difficulty to be used on the farm and the health hazard to the operators and animals limit its use in the field [5]. External Doppler ultrasonography gave accuracy rates of 83 and 93%, for the determination of singles and multiples, respectively at Days 80–95 of gestation [6]. However, considerable expertise and relatively long (5 min/ewe) examination time were required.

Real-time B-mode transabdominal ultrasonography (using a 3 or 3.5 MHz transducer) determined the fetal numbers in sheep with more than 90% accuracy between Days 50 and 100 of gestation [7–9]. However, shearing wool of the ventral abdominal wall of ewes before scanning increases the cost of labor and needs a sophisticated system for rapid handling. On the other hand there are conflicting results concerning the accuracy of the technique without pre-scanning shaving of the ventral abdominal wall [6,10,11].

Several studies have revealed that blood progesterone (P4) concentrations in ewes carrying multiples were significantly higher than those in ewes carrying singles from Week 4 until Week 20 of pregnancy [12–14]. However, the accuracy of a progesterone radioimmunoassay (P4-RIA) test to classify litter size in sheep ranged from 63 to 79% at Days 90–108 of gestation [15–17].

Ovine pregnancy-associated glycoproteins (ovPAG) or pregnancy-specific protein B (ovPSPB) are of placental origin [18] and are detectable in the maternal circulation from Week 3 of gestation till Week 2 or 3 after lambing [19,20]. Pregnancy-associated glycoprotein radioimmunoassay (PAG-RIA) or PSPB-RIA tests appeared accurate in diagnosing early pregnancy in sheep [21–23]. By using a heterologous RIA, the plasma ovPAG concentrations of ewes carrying multiples were significantly higher than those of ewes carrying singles only at Week 21 of gestation [13]. Recently a homologous RIA for ovPAG has been developed by El-Amiri et al. [24]. By using this assay,

significant differences in the levels of ovPAG were found between ewes carrying singles and those carrying multiples during the early stage of pregnancy [23].

The aim of the present study was to evaluate and compare the accuracy of transabdominal ultrasonography without pre-scanning shaving of the ventral abdominal wall, a P4-RIA and a homologous ovPAG-RIA test for the detection of fetal numbers in sheep during the second trimester of pregnancy.

2. Materials and methods

2.1. Animals, estrous synchronization and breeding

This study was carried out on pregnant Awassi–Merino crossbred ewes ($n = 100$) housed on a single farm and their ages ranged from 1.6 to 10 years. The ewes had been synchronized for estrus by using the intravaginal sponge containing 30-mg flurogestone acetate (Chrono-gest, Intervet International B.V., Boxmeer, The Netherlands) for 14 days. At the moment of sponge removal, each ewe received 1000 IU eCG (Folligon, Intervet International B.V. Boxmeer, The Netherlands) intramuscularly. All ewes were inseminated twice with fresh semen (200×10^6 spermatozoa) into the external *os* of the cervix at 48 and 56 h after sponge removal. The day of insemination was considered as Day 0 for calculating the gestational age.

2.2. Ultrasonographic examinations

All ewes were scanned transabdominally by using a real-time ultrasound scanner equipped with a 3.5 MHz linear-array transducer (Aloka SSD-500, Aloka Co. Ltd., Tokyo, Japan). The ewes were scanned two times all by the same investigator; at the first scanning the gestational ages of the ewes ($n = 100$) ranged between Days 43 and 56, while at the second scanning ($n = 81$), they ranged between Days 76 and 87, respectively. At the second scanning and sampling, 19 ewes were missed. The ewes were scanned in dorsal recumbency without shaving the ventral abdominal wall. The transducer was applied at the hairless area of the inguinal region of both sides after adding coupling gel. An animal was designated as carrying multiples if two or three fetal heads, beating hearts or independent movements of individual fetuses were recognised during ultrasonographic scanning. The time spent on each animal to reach a diagnosis was always more than 2 min.