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Original article

Evaluation of false transrectal ultrasonographic pregnancy diagnoses in sheep by measuring the plasma level of pregnancy-associated glycoproteins

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Abstract — The present study was undertaken to investigate to what extent pregnancy diagnoses made by transrectal ultrasonography could be confirmed by measurements of plasma concentration of ovine pregnancy-associated glycoproteins (ovPAG). A total of 424 Awassi × Merino ewes were synchronized for estrus and examined by transrectal ultrasonography. In Experiment 1, the ewes (n = 156) were repeatedly scanned in a standing position on d 29, 36 and 50 of gestation. Similarly, the ewes (n = 268) in Experiment 2 were scanned on d 24, 29 and 34 of gestation, but these ewes were fasted for 12 h prior to the examination and the abdominal wall of each animal was lifted up by the hands of the assistant during the scanning. Blood samples were withdrawn after each transrectal ultrasonographic examination in both experiments. Ovine PAG concentrations were measured in plasma by a heterologous radioimmunoassay and the cut-off value for pregnancy was ≥ 1 ng·mL^{-1}. Based on the lambing performance, in Experiment 1, altogether 47 false negative and 38 false positive diagnoses were made by transrectal ultrasonography in 24 and 33 ewes, respectively between d 29 and 50 of gestation. In Experiment 2, altogether 8 false negative and 13 false positive diagnoses both were made in 7 ewes between d 24 and 34 of gestation. In both experiments, all ewes with false negative diagnoses had ovPAG concentrations higher than the threshold level for pregnancy diagnosis and all ewes with false positive diagnoses had ovPAG concentrations lower than the threshold of pregnancy. Furthermore, by the PAG-RIA test all lambed or aborted ewes (n = 63) were correctly diagnosed as pregnant and with three exceptions, all non-lambed ewes (n = 361) were correctly diagnosed as non-pregnant during the examined periods of both experiments.

transrectal ultrasonography / ovPAG / pregnancy diagnosis / sheep

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1. INTRODUCTION

Real-time B-mode ultrasonography provides a simple, rapid, accurate and non-invasive means for ovine pregnancy diagnosis on the farm [1]. Ovine pregnancy (anechoic intrauterine fluid) can be diagnosed by transrectal ultrasonography (5 MHz) as early as d 17 to 19 of gestation, while the embryo proper can be imaged between d 21 to 34 of gestation [2]. However, the accuracy of transrectal ultrasonography (5 MHz) for early pregnancy diagnosis reported in several studies is rather variable. In these studies 1.2% to 87.2% of lambing ewes were incorrectly diagnosed as non-pregnant (false negative diagnoses) and 0% to 35.3% of non-lambing ewes were incorrectly diagnosed as pregnant (false positive diagnoses) before d 25 of gestation [2–4]. Even between d 25 and 50 of gestation, the percentages of false negative (0% to 35%) and false positive (0% to 17.5%) diagnoses made by transrectal ultrasonography (5MHz) were still conspicuous [2–6]. The variable results obtained in the above mentioned studies might be due to the effect of the breed and the age of the ewes [7], whether the ewes were fasted prior to scanning or not [8], the method of handling the ewes [8], the experience of the operator [9] and the incidence of embryonic or fetal mortality [7].

Ovine pregnancy-associated glycoproteins (ovPAG) are members of the aspartic proteinase family [10]. They are synthesized by the superficial epithelial layer (mono- and binucleate cells) of the fetal placenta [11]. Therefore, these glycoproteins are good indicators of a live conceptus [12]. Ovine PAG are detectable in the maternal blood from the third week of gestation until after lambing. After lambing, plasma ovPAG level decreases rapidly and becomes undetectable during the fourth week postpartum [13]. During pregnancy, the plasma ovPAG concentrations vary according to the breed of the ewe, the stage of pregnancy, and the number and genotype of the fetuses [13, 14]. Moreover, the antiserum used in the radioimmunoassay of PAG is a source of variation for PAG concentrations in the same animal [15, 16].

In spite of evidence by molecular cloning studies that there is expression of many PAG genes in the trophectoderm of ruminant placentas [11, 17], few PAG molecules have been isolated and purified. Among these glycoproteins, bovine (bo) PAG 67 KDa [18], ovPAG-1 [19], and caprine (ca) PAG 55, 59 and 62 KDa [20] are more closely related to each other both as far as their antigenic properties and their amino acid identities are concerned [10, 20].

The heterologous radioimmunoassay, using boPAG 67 KDa as the standard and tracer and antiserum raised against a mixture of ca PAG 55 + 59 KDa, accurately selected both pregnant (93.5%) and non-pregnant ewes (100%) at d 22 of gestation. The accuracy of the test to select pregnant ewes increased to 100% at d 29 of gestation [21]. Because the results of early pregnancy diagnoses obtained using transrectal, real-time, B-mode diagnostic ultrasonography with 5 MHz linear-array transducer are variable, it is necessary to evaluate the false ultrasonographic diagnoses. The present study was undertaken to investigate to what extent pregnancy diagnoses made by transrectal ultrasonography could be confirmed by measurements of plasma concentration of ovPAG on the same day.

2. MATERIALS AND METHODS

2.1. Estrus synchronization and breeding of the ewes

A total of 424 Awassi × Merino ewes, aged 1.6 to 10 y, were used in the present study. The ewes were synchronized for estrus by insertion of intravaginal sponges containing 30-mg flurogestone acetate (Chrono-gest, Intervet International B.V., Boxmeer, The Netherlands) for 14 d during

the second half of August 2000 ($n = 156$) and 2001 ($n = 268$), respectively. At the time of sponge removal, each ewe was administered 600 IU eCG (Folligon, Intervet International B.V.) 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 d 0 for calculating the gestational age.

2.2. Ultrasonographic examination

2.2.1. Experiment 1

One hundred and fifty six ewes were scanned on d 29, 36 and 50 after AI, using a real-time ultrasound scanner equipped with a 5 MHz linear-array transducer (Aloka SSD-500, Aloka Co. Ltd., Tokyo, Japan). The transducer had been modified by taping a plastic rod to the probe to control the manipulation of the transducer inside the rectum. The same operator who had been experienced for ultrasonographic pregnancy diagnosis in small ruminants carried out all the scanning. The ewes were scanned in a standing position in the milking parlor. The rectum was cleared of feces when necessary. The lubricated transducer was gently inserted into the rectum till the urinary bladder could be seen and then it was rotated 90° clockwise and 180° anti-clockwise to scan the entire reproductive tract [22]. Recognition of the allantoic fluid was considered a positive sign of pregnancy. On d 50, recognition of the fetus(es) or placentomes was used as the criterion for a positive pregnancy diagnosis. All ewes were scanned further by transabdominal ultrasonography at d 80 of gestation.

2.2.2. Experiment 2

Two hundred and sixty eight ewes were scanned at d 24, 29 and 34 after AI by the same operator using the same machine and technique applied in Experiment 1. However, the ewes were fasted for 12 h prior to scanning and their ventral abdominal wall in front of the udder was lifted up by the hands of an assistant while conducting the scanning. Recognition of the allantoic fluid was considered a positive sign of pregnancy. Ewes diagnosed pregnant ultrasonographically on d 34 were scanned further by transabdominal ultrasonography on d 50 and 80 of gestation.

At each examination in both experiments, the operator was required to record a diagnosis of either pregnancy or non-pregnancy without reference to earlier results. The number of ewes in each experiment decreased during the study periods, whereas some non-pregnant ewes returned to estrus and were re-inseminated. In addition, two pregnant ewes in Experiment 1 were missing on d 50 of gestation.

2.3. Blood sampling

After each transrectal ultrasonography, a blood sample (5 mL) was withdrawn from the jugular vein of each ewe into a heparinized vacutainer tube. Immediately (Experiment 2) or within 3 h (Experiment 1) after the collection, blood samples were centrifuged at $1500 \times g$ for 20 min. The collected plasma was stored at –20 °C till the assessment of ovPAG.

2.4. PAG radioimmunoassay

Concentrations of ovPAG on d 29, 36 and 50 (Experiment 1) and on d 24, 29 and 34 (Experiment 2) after AI were measured by a heterologous double-antibody RIA test. The boPAG 67 kDa was used as a tracer and standard, while rabbit antiserum raised against a mixture of caPAG 55 and 59 kDa (R708) was used as the first antibody. The antiserum used in this assay has been proven to be specific for PAG molecules against other members of the aspartic proteinase family (pepsinogen, pepsin, chymosin, rennet, cathepsin D and renin) [23]. The inhibition of the binding of the

tracer to the antiserum was observed with the sera of the pregnant ewes, while it was not observed with the sera of non-pregnant ewes. Therefore the assay can detect pregnancy in sheep. However, the inhibition curve generated by dilutions of the serum of pregnant ewes was not parallel to the standard curve. Thus the assay gave relative PAG concentrations which were used to differentiate between pregnant and non-pregnant ewes [21].

The procedures and the validation criteria of the assay were similar to those of Perényi et al. [24] and are described elsewhere [21]. The cut-off value of the PAG-RIA test used to detect pregnant ewes was ≥ 1 ng·mL^{-1} [21].

2.5. Data analysis

Based on the lambing performance or any other observed sign like abortion, the results of transrectal ultrasonographic examinations and the PAG test were arranged as follows: correct positive diagnosis (a), incorrect positive diagnosis (b), correct negative diagnosis (c), and incorrect negative diagnosis (d). From these values the sensitivity (a/a + d × 100), the specificity (c/c + b × 100), the positive predictive value (a/a + b × 100) and the negative predictive value (c/c + d × 100) of both tests were calculated for both experiments [25]. The exact binomial test was used to compare the sensitivity and the specificity of the transrectal ultrasonography with those of the PAG test. The same test was also used to compare the sensitivity and specificity of transrectal ultrasonography between the days of examinations in each experiment. The age of the ewes with the false negative diagnoses was compared with the age of the ewes correctly diagnosed as pregnant by transrectal ultrasonography by means of a Student *t* test [26].

3. RESULTS

The accuracies of the transrectal ultrasonography for ovine pregnancy diagnosis in Experiments 1 and 2 are shown in Tables 1 and 2, respectively.

3.1. Experiment I

Twenty-eight ewes lambed with a normal gestation length and 3 ewes aborted (after d 80) after insemination at the synchronized estrus. All lambed or aborted ewes were diagnosed as pregnant by PAG-RIA on d 29, 36 and 50. However, pregnancy was detected by transrectal ultrasonography only in 16 ewes (51.6%) on d 29, 13 ewes (41.9%) on d 36 and 15 ewes (51.7%) on d 50 of gestation (Tab. I). The level of sensitivity of the PAG-RIA test for detecting pregnant ewes was significantly ($P < 0.001$) higher than that of transrectal ultrasonography during each of the examination days. Forty-seven false negative diagnoses

Table I. Sensitivity, specificity and predictive values of transrectal ultrasonography for early pregnancy diagnosis in Awassi × Merino ewes (Experiment 1).

Days of pregnancy	No. of ewes	a	b	c	d	Se %	Sp %	+ PV %	–PV %
29	156	16	21	104	15	51.6	83.2 [e]	43.2	87.4
36	155	13	14	110	18	41.9	88.7 [f]	48.1	85.9
50	148	15	3	116	14	51.7	97.4 [e f]	83.3	89.2

a: correct positive diagnosis (pregnant), b: incorrect positive diagnosis (non-pregnant), c: correct negative diagnosis (non-pregnant), d: incorrect negative diagnosis (pregnant), Se: sensitivity, Sp: specificity, +PV: positive predictive value, –PV: negative predictive value.

Percentages within a column and with the same superscript are different ([e] $P = 0.0003$; [f] $P = 0.0127$, respectively).

Table II. Sensitivity, specificity, and predictive values of transrectal ultrasonography for early pregnancy diagnosis in Awassi × Merino ewes (Experiment 2).

Days of pregnancy	No. of ewes	a	b	c	d	Se %	Sp %	+ PV %	–PV %
24	268	26	7	229	6	81	97	78.7	97.4
29	268	31	3	233	1	96.8	98.7	91.1	99.5
34	251	31	3	216	1	96.8	98.5	91.1	99.5

a: correct positive diagnosis (pregnant), b: incorrect positive diagnosis (non-pregnant), c: correct negative diagnosis (non-pregnant), d: incorrect negative diagnosis (pregnant), Se: sensitivity, Sp: specificity, +PV: positive predictive value, –PV: negative predictive value.

(15 on d 29, 18 on d 36 and 14 on d 50) were made in 24 ewes by transrectal ultrasonography. Eight of the 24 ewes were diagnosed ultrasonographically as non-pregnant during each of the scanning days. All lambed or aborted ewes without ultrasonographic positive detection ($n = 24$) had a concentration of ovPAG higher than the threshold of pregnancy (Fig. 1). At each day of scanning, the mean age of the ewes incorrectly diagnosed as non-pregnant by transrectal ultrasonography was significantly ($P < 0.05$ d 29; $P < 0.005$, d 36 and 50) higher than that of the ewes correctly diagnosed as pregnant (Tab. III).

All non-lambing ewes except one had ovPAG concentrations lower than the threshold of pregnancy on d 29, 36 and 50 after AI. This one ewe had an ovPAG concentration slightly higher (1.02 $ng \cdot mL^{-1}$) than the threshold of pregnancy at d 29 and was ultrasonographically diagnosed non-pregnant during each of the scanning days. By transrectal ultrasonography, non-pregnancy was diagnosed in 104 ewes (83.2%) on d 29, 110 ewes (88.7%) on d 36 and 116 ewes (97.4%) on d 50 (Tab. I). The level of specificity of the PAG-RIA test was significantly ($P < 0.001$) higher than that of transrectal ultrasonography on d 29 and 36 of

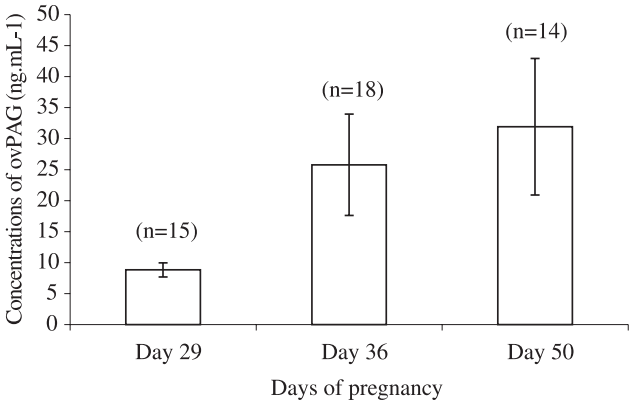

Figure 1. Mean (± S.D.) plasma concentrations of ovine pregnancy-associated glycoprotein (ovPAG) in ewes incorrectly diagnosed as non-pregnant (false negative diagnoses) by transrectal ultrasonography in Experiment 1.

Table III. Age (mean ± SD) of ewes with ultrasonographic false negative and correct positive diagnoses in different examination periods (Experiment 1).

Days of ultrasonographic examinations	Age of ewes (y)	
	Incorrect negative diagnoses	Correct positive diagnoses
29	5.3 ± 1.8 [a] (n = 15)	3.6 ± 2.3 [b] (n = 16)
36	5.3 ± 2.3 [c] (n = 18)	3.2 ± 1.5 [d] (n = 13)
50	5.6 ±1.74 [c] (n = 14)	3.3 ± 2.1 [d] (n = 15)

[a,b] $P < 0.05$.
[c,d] $P < 0.005$.

gestation. The level specificity of transrectal ultrasonography on d 50 was significantly higher than that of the test on d 29 (P = 0.0003) and 36 (P = 0.0127) of gestation (Tab. I).

Thirty-eight false positive diagnoses (21 on d 29, 14 on d 36 and 3 on d 50) were made by transrectal ultrasonography in 33 ewes. None of these ewes were incorrectly diagnosed as pregnant during each of the scanning days. All non-lambing ewes, which were ultrasonographically diagnosed as pregnant, had oPAG levels lower than the threshold of pregnancy.

3.2. Experiment 2

Twenty-nine ewes lambed with a normal gestation length and 3 ewes aborted (one after d 50 and 2 after d 80 of gestation) after insemination at the synchronized estrus. Similar to Experiment 1, all lambing or aborted ewes had ovPAG levels higher than the threshold of pregnancy during the examined periods. However, pregnancy was diagnosed by transrectal ultrasonography in 26 ewes (81%) on d 24, 31 ewes on d 29 and 34 (96.8%) of gestation (Tab. II). The level of sensitivity of the PAG-RIA test was significantly ($P < 0.05$) higher than that of transrectal ultrasonography only on d 24. Eight false negative diagnoses (6 on d 24, 1 on d 29 and 1 on d 34, respectively) were made by transrectal ultrasonography in 7 ewes during the examined period. All ewes with the false negative diagnoses had ovPAG concentrations higher than 1 ng·mL^{-1} (Tab. IV). With two exceptions, all non-lambing ewes had ovPAG concentrations lower than the threshold level established

Table IV. Concentrations of ovine pregnancy-associated glycoprotein (ovPAG) in pregnant ewes incorrectly diagnosed as non-pregnant (false negative diagnoses) by transrectal ultrasonography (US) in Experiment 2.

Ewe ID	Day 24		Day 29		Day 34	
	US	ovPAG (ng·mL^{-1})	US	ovPAG (ng·mL^{-1})	US	ovPAG (ng·mL^{-1})
A	NP	7.7	NP	10.3	P	5.5
B	NP	3.1	P	8.5	P	6.2
C	NP	5.2	P	13.5	P	7.8
D	NP	6.6	P	6.2	P	5.5
E[*]	NP	13.8	P	11.9	P	15.4
F	NP	4.9	P	6.8	P	7.0
G	P	8.1	P	16.8	NP	7.2

P: pregnant; NP: non-pregnant.
* This ewe was inseminated at the same day of the other ewes. By transrectal ultrasonography on d 29, 34 and 50, her pregnancy appeared to be older compared with other pregnancies. This ewe aborted after d 50 of gestation. It may have been conceived earlier by natural mating.

Table V. Concentrations of ovine pregnancy-associated glycoprotein (ovPAG) in non-pregnant ewes incorrectly diagnosed as pregnant (false positive diagnoses) by transrectal ultrasonography (US) in Experiment 2.

Ewe ID	Day 24		Day 29		Day 34	
	US	ovPAG (ng.mL^{-1})	US	ovPAG (ng.mL^{-1})	US	ovPAG (ng.mL^{-1})
A	P	Undetectable	P	Undetectable	P	Undetectable
B	P	Undetectable	P	Undetectable	P	Undetectable
C	P	Undetectable	P	Undetectable	NP	0.3
D	P	Undetectable	NP	Undetectable	P	0.4
E	P	Undetectable	NP	Undetectable	NP	Undetectable
F	P	Undetectable	NP	0.4	NP	0.3
G	P	Undetectable	NP	0.5	NP	0.4

P: pregnant; NP: non-pregnant.

for pregnancy. The first ewe had a high ovPAG concentration (2.3 ng·mL^{-1}) on d 24, then the level decreased to 1.5 and 1.2 ng·mL^{-1} on d 29 and 34 after AI, respectively. At the same time, this ewe was diagnosed non-pregnant by transrectal ultrasonography during the examined periods. The second ewe had an ovPAG level (1.16 ng·mL^{-1}) slightly higher than the threshold of pregnancy on d 29, while it had undetectable ovPAG levels on d 24 and 34 of gestation. This ewe was also diagnosed as non-pregnant by transrectal ultrasonography during each of the scanning days.

Thirteen false positive diagnoses (7 on d 24, 3 on d 29 and 3 on d 34, respectively) were made by transrectal ultrasonography in 7 ewes during the study period. These ewes had ovPAG levels lower than the threshold of pregnancy (Tab. V).

4. DISCUSSION

In previous studies carried out without fasting or lifting the abdomen of the animals, the accuracy of the transrectal ultrasonography (5 MHz) for diagnosing pregnancy (sensitivity) ranged from 65% to 100% between d 26 and 50 of gestation [2, 3, 4, 6]. A lower sensitivity (48.3%) of the test was obtained in our Experiment 1 between d 29 and 50 of gestation. The most probable reasons for this might be the differences in the breed and age of the ewes or in the experience of the operators [7, 9]. Forty-seven false negative diagnoses were made in 24 ewes by transrectal ultrasonography between d 29 and 50 of ges-tation. All these ewes had ovPAG concentrations higher than the threshold for pregnancy detection (≥ 1 ng·mL^{-1}). The sources of these false negative diagnoses might be the early descent of the gravid uterus into the abdominal cavity, especially in large and pluriparous ewes, then becoming out of reach of the transducer [3, 27]. This hypothesis was supported by the significant higher age of the ewes with false negative diagnoses made by transrectal ultrasonography. This source of false negative diagnosis could be less important with transabdominal ultrasonography. Also, in cows, the position of the uterus relative to the pelvic inlet has proven to influence the accuracy of transrectal ultrasonography for early pregnancy diagnosis [28]. In addition, because the ewes were not fasted prior to scanning, the intestinal gas or ingesta may have interfered with the visualization of the pregnant uterus [3, 27, 29].

The specificity (90%) of the transrectal ultrasonography for diagnosing non-pregnant ewes obtained in Experiment 1 between d 29 and 50 was in the range (82.5% to 100%) reported by other studies [2, 3, 4, 6]. Thirty-eight false positive diagnoses were made by transrectal ultrasonography in 33 ewes between d 29 and 50 of gestation. At the same time, none of these ewes had ovPAG concentrations ≥ 1 ng·mL^{-1}; indicating that the ewes were not pregnant.

The recognition of placental or fetal structures as a criterion of pregnancy precludes the possibilities of making false positive diagnoses. However, prior to d 45 of gestation, the fluid-filled vesicle is the most prominent sign of pregnancy and the fetal or the placental structures are sometimes missed during transrectal ultrasonographic examination [3]. This most likely explains the significantly higher specificity (fewer false positive diagnoses) of the transrectal ultrasonography at d 50 than that of the test at d 29 and 36 of gestation. The probable reasons for the false positive diagnoses are discussed in Experiment 2.

In Experiment 2, the overall sensitivity (92%) of the transrectal ultrasonography for detecting pregnant ewes was higher than that (87%) obtained in another study between d 25 and 50 of gestation [5]. However, the specificity of the test for detecting non-pregnant ewes in both studies were the same. In that study, the ewes were fasted for 12 h before the ultrasonographic examinations, but the abdominal wall of the animal was not lifted up during scanning as in our study. In our Experiment 2, 6 false negative diagnoses were made by transrectal ultrasonography at d 24 of gestation. All of them had ovPAG concentrations higher than the threshold of pregnancy. On d 20 to 25 of gestation, the amount of embryonic fluid is small, and therefore it is difficult to be detected by a 5 MHz linear-array transducer, especially in large and mature ewes [2, 3]. With one exception, these false negative ultrasonographic diagnoses were confirmed as correct positive ones by examining the ewes on d 29 and 34 of gestation. Therefore these results emphasize the importance of the re-examination of ewes with negative transrectal ultrasonographic diagnoses performed before day 29 of gestation.

Seven false positive diagnoses were made by transrectal ultrasonography on d 24 of gestation; three of them were also incorrectly diagnosed as pregnant on d 29 and 34 of gestation. All seven ewes had ovPAG concentrations lower than the threshold level for pregnancy. This also confirms that these ewes were non-pregnant at the time of scanning. Metritis, pyometra and hydrometra or any unknown conditions associated with the accumulation of anechoic intrauterine or abdominal fluid might be the reason for the false positive diagnoses made by transrectal ultrasonography in both experiments [29–31].

Regarding the PAG-RIA test, all lambed or aborted ewes had ovPAG concentrations higher than the threshold level for pregnancy in both experiments. In addition, with three exceptions, all non-pregnant ewes had undetectable ovPAG levels or lower than the threshold levels for pregnancy during the examined periods in both experiments. One ewe was diagnosed as non-pregnant by ultrasonography on d 24 of gestation and had ovPAG levels (2.3 ng·mL^{-1}) higher than the threshold of pregnancy, but it was lower than the average ovPAG concentration (5.8 ± 2.3 ng·mL^{-1}) for that day. Thereafter, the ovPAG concentration decreased to 1.2 ng·mL^{-1} on d 29 and 1.1 ng·mL^{-1} on d 34, respectively. Embryonic mortality might have occurred in this ewe before d 24 of gestation. The other two ewes had ovPAG levels only just above the threshold level on d 29.

Similar to the results reported in our previous study [8], fewer false negative and positive diagnoses were made by transrectal ultrasonography in Experiment 2 than in Experiment 1. The probable reasons may be the effect of the fasting and/or lifting the abdomen of the animals performed in

Experiment 2. Fasting ewes for 12 to 24 h prior to the scanning reduces the intestinal gases or ingesta, which might interfere with the identification of the pregnant uterus or induce image artifacts [3, 29]. In addition, lifting the abdomen of the ewes by the hand of the assistant may push the reproductive tract, especially in large breeds to the pelvis to be within reach of the ultrasound beam.

Compared to the P4 test, the PAG RIA test is more specific because it can differentiate between pregnancy and prolonged inter-estrus intervals [21]. In addition, unlike the P4 test, the timing of blood sampling for the PAG-RIA test is not dependent on the knowledge of the exact estrus. The PAG-RIA test is more accurate than the transrectal ultrasonography for early pregnancy diagnosis (d 24). However, transrectal ultrasonography has the advantage over PAG-RIA of being an on-farm test. Currently, efforts are being made to develop ELISA kits for PAG to enable the producer to apply the test on the farm.

In conclusion, the accuracy of transrectal ultrasonography for detecting early pregnancy in sheep can be evaluated by measurement of plasma ovPAG concentrations. The heterologous PAG-RIA is more accurate than tranrectal ultrasonography for diagnosing pregnant and non-pregnant Awassi × Merino ewes on d 24 of gestation. Furthermore, transrectal ultrasonography is an accurate method for pregnancy diagnosis after d 24 of gestation when the Awassi × Merino ewes are fasted before scanning and their abdominal wall is lifted up during the scanning.

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REFERENCES

[1] Cunningham W, Marsh D. Pregnancy diagnosis. In: Youngquist RS (Ed), Current Therapy in Large Animal Theriogenology, WB Saunders Company, Philadelphia, 1997, p 612–616.

[2] Garcia A, Neary MK, Kelly GR, Pierson RA. Accuracy of ultrasonography in early pregnancy diagnosis in the ewe. Theriogenology 1993, 39: 847–861.

[3] Gearhart MA, Wingfield WE, Knight AP, Smith JA, Dargatz DA, Boon JA, Stockes CA. Real-time ultrasonography for determining pregnancy status and viable fetal numbers in ewes. Theriogenology 1988, 30: 323–337.

[4] Kaulfuss KH, Zipper N, May J, Suss R. Ultrasonic pregnancy diagnosis (B-mode) in sheep. 2. Comparative studies using transcutaneous and transrectal pregnancy diagnosis. Tierärztl Prax 1996, 24: 559– 566.

[5] Buckrell BC, Bonnett BN, Johnson WH. The use of real-time ultrasound rectally for early pregnancy diagnosis in sheep. Theriogenology 1986, 25: 665–673.

[6] Kähn W, Achtzehn J, Kähn B, Richter A, Schulz J, Wolf M. Sonography of pregnancy in sheep. II. Accuracy of transrectal and transcutanous pregnancy diagnosis. Dtsch Tierarztl Wochenscher 1993, 100: 29–31.

[7] Buckrell BC. Application of ultrasonography in reproduction in sheep and goats. Theriogenology 1988, 29: 71–84.

[8] Karen A, Szabados K, Beckers JF, Szenci O. Accuracy of transrectal ultrasonography for determination of early pregnancy in sheep: Effect of fasting and handling of animals. Cairo: Proc. 53rd EAAP Annual Meeting, 2002, p 115.

[9] Bretzlaff K, Romano JE. Advanced reproductive techniques in goats. Vet Clin North Am Food Anim Pract 2001, 17: 421–434.

[10] Xie S, Low BG, Nagel RJ, Kramer KK, Anthony RV, Zoli AP, Beckers JF, Roberts RM. Identification of the major pregnancy-specific antigens of cattle and sheep as inactive members of aspartic proteinase family. Proc Natl Acad Sci USA 1991, 88: 10247–10251.

[11] Green JA, Xie A, Quan X, Banga B, Gan X, Mathialagan N, Beckers JF, Roberts MR. Pregnancy-associated bovine and ovine glycoproteins exhibit spatially and temporally distinct expression patterns during pregnancy. Biol Reprod 2000, 62: 1624–1631.

[12] Zarrouk A, England I, Sulon J, Beckers JF. Determination of pregnancy-associated glycoprotein concentrations in goats (*Capra hircus*) with unsuccessful pregnancies: a retrospective study. Theriogenology 1999, 51: 1221–1231.

[13] Ranilla MJ, Sulon J, Carro MD, Mantecon AR, Beckers JF. Plasmatic profiles of pregnancy–associated glycoprotein and progesterone levels during gestation in Churra and Merino sheep. Theriogenology 1994, 42: 537–545.

[14] Ranilla MJ, Sulon J, Mantecon AR, Beckers JF, Carro MD. Plasma pregnancy-associated glycoprotein and progesterone concentrations in pregnant Assaf ewes carrying single and twin lambs. Small Rumin Res 1997, 24: 125–131.

[15] González F, Sulon J, Garbayo JM, Batista M, Cabrera F, Calero PO, Gracia A, Beckers JS. Early pregnancy diagnosis in goats by determination of pregnancy-associated glycoprotein concentrations in plasma samples. Theriogenology 1999, 52: 717–725.

[16] González F, Sulon J, Garbayo JM, Batista M, Cabrera F, Calero PO, Gracia A, Beckers JS. Secretory profiles of pregnancy-associated glycoproteins at different stages of pregnancy in the goat. Reprod Domest Anim 2000, 35: 79–82.

[17] Garbayo JM, Green JA, Manikkam M, Beckers JF, Keisling DO, Ealy AD, Roberts MR. Caprine pregnancy associated glycoproteins (PAG): Their cloning, expression, and evolutionary relationship to other PAG. Mol Reprod Dev 2000, 57: 311–322.

[18] Zoli AP, Beckers JF, Wouters-Ballman P, Closset J Falmagne P, Ectors F. Purification and characterization of a bovine pregnancy-associated glycoprotein. Biol Reprod 1991, 45: 1–10.

[19] Zoli AP, Beckers JF, Ectors F. Isolation and partial characterization of an ovine pregnancy associated glycoprotein. Ann Med Vet 1995, 139: 177–184.

[20] Garbayo JM, Remy B, Alabart JI, Folch J, Wattiez R, Falmange P, Beckers JF. Isolation and partial characterization of a pregnancy-associated glycoprotein family from the goat placenta. Biol Reprod 1998, 58: 109–115.

[21] Karen A, Beckers JF, Sulon J, Sousa NM, Szabados K, Reczigel J, Szenci O. Early pregnancy diagnosis in sheep by progesterone and pregnancy-associated glycoprotein tests. Theriogenology 2003, 59: 1941–1948.

[22] Schrick FN, Inskeep EK. Determination of early pregnancy in ewes utilizing transrectal ultrasonography. Theriogenology 1993, 40: 295–306.

[23] Perényi ZS, Szenci O, Drion PV, Banga-Mboka H, Sousa NM, El-Amiri B, Beckers JF. Aspartic proteinase members secreted by the ruminant placenta: Specificity of three different radioimmunoassay systems for measurement of pregnancy-associated glycoproteins. Reprod Domest Anim 2002, 37: 324–329.

[24] Perényi ZS, Szenci O, Sulon J, Drion PV, Beckers JF. Comparison of the ability of three radioimmunoassay to detect pregnancy-associated glycoproteins in bovine plasma. Reprod Domest Anim 2002, 37: 100–104.

[25] Martin SW, Meek AH, Willeberg P. Veterinary Epidemiology, Principles and Methods. Iowa State University Press, Iowa, 1987.

[26] Armitage P, Berry G. Statistical Methods in Medical Research. Blackwell Science Ltd, London, 1998.

[27] Bretzlaff K, Edwards J, Forrest D, Nuti L. Ultrasonographic determination of pregnancy in small ruminants. Vet Med 1993, 88: 12–24.

[28] Szenci O, Gyulay GY, Nagy P, Kovács L, Varga J, Taverne MAM. Effect of uterine location relative to the pelvic inlet on the accuracy of early bovine pregnancy diagnosis by means of ultrasonography. The Vet Quart 1995, 17: 37–39.

[29] Sharky S, Callan RJ, Mortimer R, Klimberling C. Reproductive techniques in sheep. Vet Clin North Am Food Anim Pract 2001, 17: 235–255.

[30] Bretzlaff KN. Development of hydrometra in a ewe flock after ultrasonography for determination of pregnancy. J Am Vet Med Assoc 1993, 203: 122–125.

[31] Kähn W. Ultrasonography in sheep and goats. In: Veterinary Reproductive Ultrasonography, Mosby-Wolf, London, 1994, p 187–210.