

A NEW TEST FOR EARLY PREGNANCY DIAGNOSIS IN SHEEP: DETERMINATION OF OVINE PREGNANCY ASSOCIATED GLYCOPROTEIN (ovPAG) CONCENTRATION BY MEANS OF A HOMOLOGOUS RADIOIMMUNOASSAY

*Een nieuwe test voor vroege drachtdiagnose bij schapen:
bepaling van Oviene Pregnancy-Associated Glycoproteïnen(ovPAG) concentratie
door middel van een homologe radioimmunoassay*

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ABSTRACT

Early pregnancy diagnosis is a useful tool in the management of sheep breeding. In this study different methods for pregnancy diagnosis in sheep, including the assessment of ovine Pregnancy-Associated Glycoproteins (ovPAGs), are briefly discussed. Subsequently, in a field trial the use of transrectal ultrasound is compared with the assessment of the concentration of ovPAGs for early pregnancy diagnosis.

At the start of the breeding season in 2002, 192 Texel ewes at 4 different farms were synchronized and afterwards mated or artificially inseminated (AI). At the moment of synchronization (D-14) and at 25 (D25), 35 (D35) and 45 (D45) days after mating/AI, transrectal ultrasound was performed and blood was collected by jugular vein puncture. The ovPAG concentration in the plasma of the ewes was measured by means of a homologous radioimmunoassay (RIA).

The specificity of transrectal ultrasound was 85%, and the sensitivity was 92% at D25, 94% at D35 and 95% at D45. The specificity of the homologous RIA was 100%, and the sensitivity was 99% at D25, and 100% at D35 and D45. Higher ovPAG concentrations were detected at D35 and D45 than at D25 ($P < 0.01$). An interesting finding was that within the same breed (Texel), ovPAG concentrations were significantly affected by the farm ($P < 0.01$).

In conclusion, determination of ovPAG concentration by means of homologous RIA can be used for early pregnancy diagnosis in sheep and is more reliable than transrectal ultrasound at 25, 35 or 45 days of gestation.

SAMENVATTING

Vroege drachtdiagnose maakt deel uit van een optimaal management op het schapenbedrijf. In deze studie worden de verschillende methoden voor drachtdiagnose bij schapen kort belicht. In een aansluitende veldproef worden twee methoden voor vroegtijdige drachtdiagnose met elkaar vergeleken: 1. transrectale real time echografie (5MHz, lineaire sonde) en 2. het meten van 'ovine Pregnancy-Associated Glycoproteïnen' (ovPAG) met behulp van een homologe Radioimmunoassay (RIA).

Bij aanvang van het dekseizoen in 2002 werden 192 Texel oaien van 4 verschillende bedrijven gesynchroniseerd met behulp van intravaginale sponzen. Vervolgens werden de oaien gedekt of kunstmatig geïnsemineerd. Bloedafnames voor ovPAG-bepaling werden verricht op het moment van het inbrengen van de sponzen (D-14) en

op 25 (D25), 35 (D35) en 45 (D45) dagen na dekking of kunstmatige inseminatie. Op D25, D35 en D45 werden de oöien eveneens aan een drachtonderzoek met behulp van transrectale echografie onderworpen.

De specificiteit voor transrectale echografie was 85% en de sensitiviteit 92%, 94% en 95% op respectievelijk D25, D35 en D45. De specificiteit van de homologe RIA was 100% en de sensitiviteit was 99% op D25 en 100% op D35 en D45. De ovPAG-concentraties bij drachtige oöien waren significant hoger op D35 en D45 dan op D25 ($P < 0,01$). Een interessante bevinding was dat niettegenstaande het allemaal oöien van eenzelfde ras (Texel) waren, er een significant verschil in ovPAG-concentratie werd gevonden tussen de bedrijven ($P < 0,01$).

Uit dit onderzoek kan besloten worden dat de bepaling van de ovPAG-concentratie kan gebruikt worden voor vroege drachtdiagnose bij schapen en dat de bepaling van de ovPAG-concentratie met een homologe RIA betrouwbaarder is dan de transrectale echografie op 25, 35 of 45 dagen dracht.

INTRODUCTION

In Belgium, sheep breeding is of little importance economically. According to official data, only about 150,000 ewes or 0.15% of the total number of European sheep are bred in Belgium. In Belgium, 41,667 farms are registered, with an average flock size of 5.8 sheep (Sanitel data, March 2002). This implies that most of the Belgian sheep breeders are hobby breeders. Only on 34 farms are sheep kept on a commercial basis. On 9 of these farms, sheep are kept for milk production, mostly as a subsidiary activity in addition to cattle breeding or agriculture (A. Tylleman, personal communication, 2003). In Belgium it is not possible to earn a living from raising sheep for meat or wool production. Eighty percent of the lamb meat that is consumed in Belgium is imported, with New Zealand as the main importer.

Due to the high prices of land and the relatively low value of sheep, sheep breeding is not a profitable sector in Belgium. Extra environmental restrictions enforced by the government and all the paper work that is required to meet the legal commitments inhibit the expansion of the existing sheep farms. To make the existing sheep farms profitable, good flock management is required.

Winter is the most expensive period of the year for sheep breeders. During this period the costs of maintenance and the incidence of disorders and related treatment costs are highest. Separation of pregnant and non-pregnant ewes might reduce the losses due to abortions, stillbirths and the production of weak lambs (Wani *et al.*, 1998). Non-pregnant ewes can be sold, thus reducing the feeding and medical expenses, while non-pregnant lambs can be marketed at higher prices than they would bring as mature ewes (Gearhart *et al.*, 1988). Separation of the pregnant ewes in accordance with their stage of gestation and the number of lambs expected allows for appropriate nutritional management, the prevention of pregnancy tox-

mia (Ford, 1983), the minimization of prelambling feeding costs, the reduction of the incidence of dystocia (Gearhart *et al.*, 1988), and the optimization of the birth weight, weaning weight and survivability of the lambs.

Research by Kaulfuß *et al.* (1999) showed that in farms with more than 300 ewes and an average litter size of 1.3 lambs, early pregnancy diagnosis was profitable if the pregnancy rates were lower than 97%. In small farms, where sheep are bred for hobby, the economic aspect is less important. Hobby breeders are curious and too impatient to wait until parturition to know the outcome of their breeding program. Top breeders also buy and sell pregnant ewes for relatively high prices. In these cases certificates are required to confirm pregnancy, which means that pregnancy needs to be detected with a high specificity and sensitivity. Determination of the concentration of ovine Pregnancy Associated Glycoproteins (ovPAGs) in the blood of the ewe by means of a homologous radioimmunoassay (RIA) as described by El Amiri *et al.* (2003a, 2003b) is a detection method that would fulfil these requirements.

In this study, the different methods which can be used in the field for pregnancy diagnosis in sheep are briefly discussed, and in a field trial the transrectal ultrasound method is compared with the assessment of the ovPAG concentration for early pregnancy diagnosis.

METHODS FOR PREGNANCY DIAGNOSIS IN SHEEP

The ideal method for pregnancy diagnosis needs to be cheap, fast, reliable and safe, and it must be applicable under field conditions. During the last fifty years several methods have been developed to detect pregnancy, though most of them are seldom used due to a lack of reliability or handiness under field conditions. These methods include radiography (Ford *et*

al., 1963), laparotomy (Lamond, 1963), rectal abdominal palpation (Hulet, 1972), vaginal cytology (Richardson, 1972), the rosette inhibition test (Ishwar, 1995), estrone sulphate concentration (Tsang, 1978), A-mode ultrasound (Meredith and Madani, 1980) and Doppler ultrasound (Fraser and Robertson, 1967). The more frequently used methods include detection of non-return to estrus by a ram provided with a harness, determination of progesterone concentration (McPhee and Tiberghien, 1987), abdominal palpation (Pratt and Hopkins, 1975), and transrectal and transabdominal real time ultrasonography. Recently a new test for the determination of ovine Pregnancy Associated Glycoproteins (ovPAGs) by means of a homologous radioimmunoassay (RIA) has been developed (El Amiri *et al.*, 2003b, 2003c).

Non-return to estrus

One of the most common methods for pregnancy diagnosis in sheep is the non-return to estrus. By introducing a ram fitted with a harness with a marker, the mated ewes are marked. By changing the color of the marker every 16 days, a distinction can be made between the ewes that are pregnant from the first mating and the ewes that come back into estrus. The sensitivity (pregnant ewes detected as pregnant) of this detection method is high (94%), while the specificity (non-pregnant ewes detected as not pregnant) is rather low (62%) (Verberckmoes *et al.*, 2001). Pathological changes in the uterus or ovaries, as well as physiological anestrus at the end of the breeding season can be responsible for false positive results (not marked, and not pregnant). It is also known that even pregnant ewes are sometimes marked by the ram, though exact figures are not available. The major advantages of this detection method are the low cost, the simplicity and the high reliability within the breeding season.

Progesterone concentration

In the breeding season, ewes come into estrus every 16-17 days. During the estrous cycle, progesterone concentrations are low from 2 days before until 4 days after ovulation. In non-pregnant ewes the highest progesterone concentration is reached at day 14, and it then drops to a concentration of less than 1 ng/mL at day 17. Simultaneously with the drop in progesterone, the estrogen concentration increases and a new ovulation is induced (Mukasa-Mugerwa and Viviane, 1992). However, when sheep are pregnant,

the corpus luteum develops into a corpus luteum graviditatis, and the progesterone concentration remains high. After 50 days of pregnancy, the production of progesterone by the corpus luteum graviditatis is taken over by the placenta (Fitzpatrick, 1986). Increased progesterone concentrations at 16 to 20 days after mating can be used for early pregnancy detection (Musaka-Mugerwa and Viviane, 1992). Enzyme linked immunoassay (ELISA) as well as radioimmunoassay (RIA) can be used for determination of the progesterone concentration, with the threshold level for pregnancy varying between 1 and 1.9 ng/mL (Susmel and Piasentier, 1992; Karen *et al.*, 2003). However, in practice the exact moment of mating is seldom known and blood sampling between 16 and 20 days after mating is not always feasible. When blood sampling is performed, arbitrary and increased progesterone concentrations are observed; this can be due either to pregnancy or to the presence of an active corpus luteum in non-pregnant ewes. Notwithstanding the high sensitivity of the progesterone assay, a lot of false positive results are obtained, resulting in a low specificity (Susmel and Piasentier, 1992). In the second half of gestation, higher progesterone concentrations are observed in ewes pregnant with multiples than in those pregnant with singles, while no effect of the sex of the lambs on progesterone concentration could be found (Kalkan *et al.*, 1996).

Abdominal palpation

Two methods have been developed for pregnancy diagnosis in sheep by means of palpation: rectal abdominal palpation and abdominal palpation. When rectal abdominal palpation is performed, the ewe is fixed and a rigid tube is introduced in the rectum. When a mass is felt between the abdominal wall and the tube, the ewe is considered to be pregnant. Notwithstanding the fact that the accuracy of this technique is 95% between 65 and 110 days of gestation, it is too labor-intensive and hazardous (Turner and Hindson, 1975). In contrast to rectal abdominal palpation, abdominal palpation is a more feasible detection technique. This can be performed rapidly (150-200 ewes/h) on ewes in a standing position with an accuracy of 80 to 95% between 90 and 130 days of pregnancy (Pratt and Hopkins, 1975). Pregnancy detection is performed by pushing with one hand on the left side of the abdomen, while the other hand is used for palpating the foetus from the right groin side. Despite the fact that this technique can only be used for pregnancy detection in a late stage of gestation and the fact that some

experience is required, it is very cheap, easy to perform and not time consuming.

Transrectal and transabdominal real time ultrasonography

Transrectal ultrasonography is performed on ewes in a standing position with a linear probe of 5 or 7MHz, which is fixed in a stick. After putting lubricant on the top of the stick, it is introduced into the rectum. The bladder is the ideal orientation point, being visible as a triangle when filled with urine. From 25 days of gestation onwards, the uterus filled with fluid can be detected cranially from the bladder. From 27.6 ± 2.4 days of gestation onwards, placentomes and embryos can be diagnosed (Buckerell *et al.*, 1986; Garcia *et al.*, 1993). When the ewe is not pregnant, no uterus can be detected. False positive diagnoses can be obtained as a result of embryonic mortality or abortion. Occasionally, the bladder, pyometra or hydrometra can be mistakenly diagnosed as a pregnant uterus. However, in the latter cases no fetuses or placentomes are present (Gearheart *et al.*, 1988; Haibel, 1990). Before 25 days of gestation, the number of false negative diagnoses is high. This can be due to the limited amount of fluid present in the uterus or the inaccessibility of the reproductive tract for scanning. The former problem is especially encountered in older ewes of large size in which the reproductive organs are sagged in the abdominal cavity (Buckerell *et al.*, 1986). In these ewes, pregnancy can be diagnosed by means of transabdominal ultrasound.

When transabdominal ultrasound is performed, a probe of 3 to 5 MHz is placed on the groin side of the ewe. Lubricant is used to maximize the contact between probe and skin. With this technique, no reliable results can be obtained before 35 days of gestation. The ideal period for transabdominal ultrasound is between 45 and 90 days of gestation. At this stage, pregnancy can be diagnosed with a sensitivity and specificity of 98 and 100%, respectively, and the number of fetuses can be predicted by an experienced investigator with high accuracy. However, since evaluation of the number of lambs is time consuming, it decreases the examination speed. At a later stage of gestation, only parts of fetuses can be observed and no accurate prediction of the number of fetuses can be made (White *et al.*, 1984; Gearheart *et al.*, 1988; Haibel, 1990).

When transrectal ultrasound is used for pregnancy diagnosis, 40-60 ewes/h can be examined for pregnancy, while 60-350 ewes/h can be examined when

transabdominal ultrasound is used (Garcia *et al.*, 1993; Kaulfuß *et al.* 1999). The high variation in speed of pregnancy detection is largely due the organization at the farm, the number of assistants, and the experience of the investigator. The best conditions for pregnancy detection in a flock are obtained when the ewes are examined later than 35 days after ram removal, in a dark area with an ultrasound instrument of good quality.

Ovine Pregnancy Associated Glycoproteins (ovPAG)

Pregnancy Associated Glycoproteins (PAGs) are produced by binucleate cells of the trophectoderm (Xie *et al.*, 1991) and have been diagnosed in the blood of pregnant cattle (Zoli *et al.*, 1991), sheep (Zoli *et al.*, 1990; El Amiri *et al.*, 2002) pigs (Szafarska *et al.*, 1995), deer (Osborn *et al.*, 1996), goats (Garbayo *et al.*, 1997), dogs (Gan *et al.*, 1997), horses, zebras (Green *et al.*, 1999) and zebus (Sousa *et al.*, 2000). Determination of the PAG concentration is performed by means of an RIA. At first, only a heterologous RIA was available for determination of the PAG concentration in sheep (Ranilla *et al.*, 1994), but recently a homologous RIA has been developed (El Amiri *et al.*, 2003a, 2003b). In a homologous RIA, all substances (sample, tracer, standard) used to determine the PAG concentration originate from the same species, while in a heterologous RIA, the sample originates from a different species than the other substances used for the RIA. However, since rabbit antibodies against bovine PAGs also bind ovine PAGs, they can be used for determination of the ovPAG concentration in a heterologous RIA. Small scaled studies have shown that pregnancy in sheep can be detected by determination of the PAG concentration in the plasma of the ewes from 3 weeks of pregnancy onwards (Ranilla *et al.*, 1994). There is some evidence that the concentration of ovPAG is affected by the fetal number and the sex of the fetus. Ranilla *et al.* (1994; 1997) found that from week 12 of gestation until lambing, higher PAG concentrations were observed in ewes carrying two fetuses than in ewes carrying a single, and that during the last 3 weeks of gestation higher PAG concentrations were observed in ewes carrying male fetuses than in ewes carrying female fetuses. The sensitivity and the specificity of the homologous RIA for pregnancy detection in cattle are 93% and 98% respectively at 35 days of gestation (Zoli *et al.*, 1992). In this study, the sensitivity and the specificity of the homologous RIA for

pregnancy detection in sheep are examined for the first time at three different times early in gestation.

MATERIALS AND METHODS

At the start of the breeding season 192 Texel ewes at 4 different farms were synchronized by means of intra-vaginal sponges impregnated with 40 mg cronolone (Chronogest[®], Intervet, Boxmeer, Netherlands). After 12 days, the sponges were removed and 400-500 IU of equine chorionic gonadotropin (Folligon[®], Intervet, Boxmeer, Netherlands) was administered (im). The ewes were mated or artificially inseminated at 48 to 55 hrs after sponge removal.

Blood samples for pregnancy diagnosis by means of ovPAG determination were taken at the moment of sponge insertion (D-14), and at 25 (D25), 35 (D35) and 45 (D45) days after insemination. At least 6 mL of blood was collected by means of jugular vein puncture in a tube with lithium-heparine (Venoject, Terumo Europe NV, Leuven, Belgium). After 10 min of centrifugation at 1620 x g, the plasma was aspirated, divided over 2 Eppendorf tubes, and stored at -20°C until analysis.

The ovPAG concentrations were determined by means of a homologous RIA. Radioactively labelled ovPAG was used as tracer, and antibodies of rabbits immunized with ovPAGs with a molecular weight of 57 and 59kD were used as antiserum. The ovine PAG was weighed and diluted to generate standard points ranging from 0.4 to 100 ng/ml. The threshold level for pregnancy was 2 ng/mL.

At D25, D35 and D45, the ewes were also examined for pregnancy by means of transrectal ultrasound (5MHz, linear probe). A ewe was considered pregnant when fluid was detected in the uterus. A mixed

model analysis with ewe as random effect and moment of gestation as fixed effect (SAS) was used. To determine the specificity of the two detection methods, the ewes were assessed as being not pregnant at the moment of sponge insertion, and at D25 and D35 if they lambbed 164 ± 5 days after sponge removal. These ewes, which were assumed to be fertilized at the second estrus after sponge removal (D18), consequently had to have a negative result for pregnancy detection with both methods at D25 and D35. To determine the sensitivity of the two detection methods, the ewes lambing at 147 ± 5 days after sponge removal were assessed as being pregnant at D25, 35 and 45.

RESULTS

The average ovPAG concentration at D-14 was 0.30 ± 0.15 ng/mL, with 0.20 ng/mL and 1.15 ng/mL as minimum and maximum concentrations. The threshold level for pregnancy was fixed at 2 ng/mL (Verberckmoes *et al.*, 2003). The average ovPAG concentration in the pregnant ewes was 7.16 ± 2.70 ng/mL, 9.07 ± 2.72 ng/mL and 8.44 ± 2.71 ng/mL at D25, D35 and D45, respectively. The ovPAG concentrations were significantly higher at D35 and D45 than at D25 ($P < 0.01$). A significant difference in ovPAG concentration was observed between pregnant ewes of different farms ($P < 0.01$) (Table 1).

The sensitivity of this test was 99% at D25 and 100% at D35 and D45, while the specificity of the ovPAG concentration for detection of pregnancy was 100%.

When transrectal ultrasound was performed at D25, several cross-sections through the uterus filled with fluid and embryos were sometimes observed in pregnant ewes (Fig 1a). From D35 onwards placentomes

Table 1. Difference in ovPAG concentration (mean \pm SD) in ng/mL at D14, D25, D35, and D45 in pregnant ewes of different farms.

	D14 mean \pm SD	D25 mean \pm SD	D35 mean \pm SD	D45 mean \pm SD	Average mean \pm SD
Farm 1 (n = 38)	0.22 \pm 0.03	6.79 \pm 2.40	7.65 \pm 2.67	7.68 \pm 2.28	5.13 \pm 0.63
Farm 2 (n = 41)	0.44 \pm 0.22	7.64 \pm 2.02	10.25 \pm 2.21	10.38 \pm 3.30	7.08 \pm 0.63
Farm 3 (n = 37)	0.28 \pm 0.10	7.50 \pm 3.13	9.41 \pm 3.97	7.80 \pm 3.96	5.97 \pm 0.64
Farm 4 (n = 26)	0.24 \pm 0.08	6.53 \pm 2.08	9.38 \pm 3.70	7.94 \pm 3.50	5.74 \pm 0.73

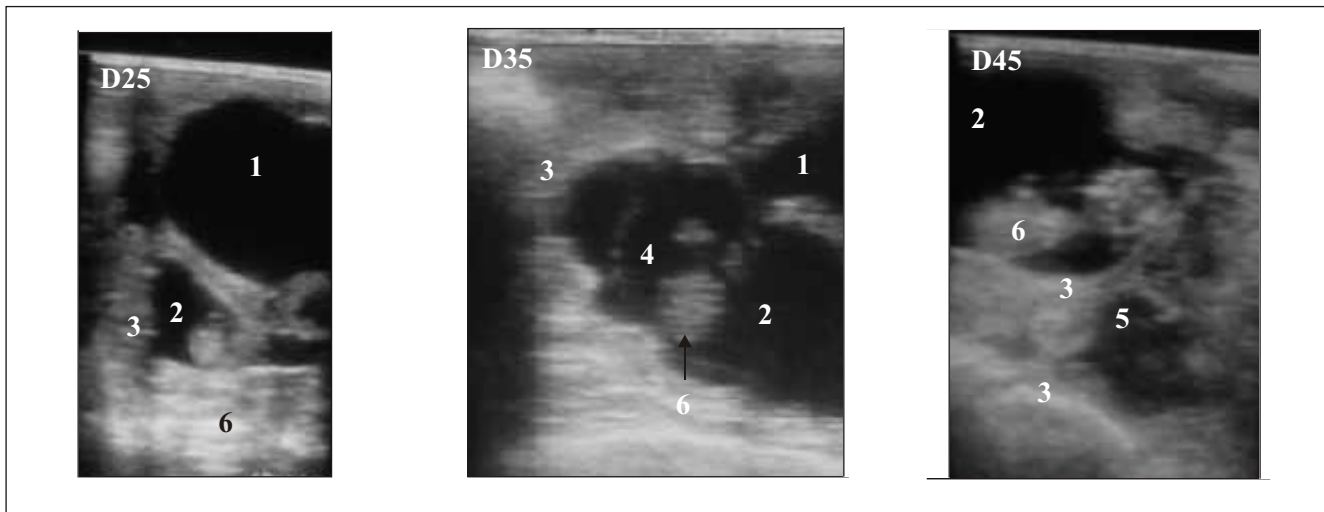


Fig 1. Images obtained by transrectal ultrasound in ewes at 25, 35 and 45 days of gestation. 1 = bladder, 2 = amniotic/allantoic fluid, 3 = uterine wall, 4 = amniotic fluid, 5 = placenta 6 = embryo/fetus.

could also be observed (Figs. 1b and 1c). In doubtful cases, the ewes were classified as being not pregnant. The sensitivity was 92% at D25, 94% at D35 and 95% at D45, and the specificity of transrectal ultrasound was 85%.

DISCUSSION

In this study, a field trial concerning pregnancy detection by means of determination of the ovPAG concentration with a homologous RIA has been described for the first time. One interesting finding was that a significant difference in ovPAG concentration was observed between pregnant ewes of different farms. Since ovPAGs are produced by binucleate cells of the trophoblast, it is likely that factors affecting the placental mass also affect the ovPAG concentration in the blood of the ewe. Wallace *et al.* (1997) found a correlation between placental mass and the pregnancy associated glycoprotein (PSPB), which later turned out to be a pregnancy associated glycoprotein. The main factor influencing the placental mass is the number of lambs (Alexander, 1974). At 21 weeks of pregnancy, higher ovPAG concentrations have been measured in ewes pregnant with multiples compared with those pregnant with singletons (Ranilla *et al.*, 1997). However, in our study the average litter size did not differ between the different farms and, moreover, pregnancy was detected at a very early stage of gestation, at which time the total placental mass cannot be very different between ewes pregnant with singles and ewes pregnant with multiples. A second factor that can affect the placental mass is the nutritional treatment of the ewes. Wallace *et al.* (1997) showed that PSPB

concentrations were higher in young adolescent ewes receiving a low proportion of a complete diet than in ewes receiving a high proportion of a complete diet. The higher food intake induced a significant increase in growth of the adolescent ewes, though it had a negative effect on the placental growth. In our study, the body condition of the ewes at farm 2 (data not shown) was clearly lower than at the other farms, and higher ovPAG concentrations were obtained (Table 1). This finding confirms the hypothesis that differences in nutrition and related body condition between ewes at the different farms may be responsible for the greatest part of variation in ovPAG concentrations between these farms.

In addition, genetic differences at the farm level may result in different live weights, condition scores and fetal placental masses, and thus indirectly affect the ovPAG concentrations (Kaulfuß *et al.*, 2000). Although sheep probably possess more than 100 genes, many of which are placentally expressed, genes with a direct effect on ovPAG concentrations have not yet been identified (Xie *et al.*, 1997).

To determine the accuracy of the pregnancy detection methods in ewes, the sensitivity (pregnant ewes detected as pregnant) and specificity (non-pregnant ewes detected as not pregnant) were determined. The sensitivity of the homologous RIA used in this field trial was 99% at D25, and 100% at D35 and D45. This means that at D25, 99% of the lambing ewes and at D35 and D45 all of the lambing ewes had ovPAG concentrations higher than 2 ng/mL. The specificity of the RIA was 100%. This means that all non-pregnant ewes had ovPAG concentrations lower than 2 ng/mL (with 100% confidence).

The absolute ovPAG values in this study obtained with the homologous RIA at D25 were on average 3 ng/mL higher than those reported by Karen *et al.* (2003) at D22, who used a heterologous RIA. This can be due partly to the increase in ovPAG concentration between D22 and D25, but also to the higher sensitivity of the homologous RIA, or to the differences in breed and management at different farms.

Compared with transrectal ultrasound, the determination of ovPAG concentration turned out to be a more reliable tool for early pregnancy diagnosis. Both the sensitivity and the specificity of the homologous RIA were higher than those obtained with transrectal ultrasound. The sensitivity of the transrectal ultrasound obtained in our study was even higher than the 65% sensitivity between 25 and 50 days of pregnancy reported by Gearhart *et al.* (1988) and Garcia *et al.* (1993). In older ewes of large breeds, the chance that the pregnant uterus has sagged into the abdominal cavity and is therefore unreachable for pregnancy diagnosis by transrectal ultrasound is greater than in younger ewes and ewes of small breeds. When the pregnant uterus has sagged into the abdominal cavity, pregnancy can be diagnosed more easily with transabdominal than with transrectal ultrasound. However, transabdominal ultrasound was not performed in this field trial because pregnancy diagnosis with this technique is not reliable before 35 days of gestation (Kaulfuß *et al.*, 1996). The ideal period for pregnancy detection by means of transabdominal ultrasound is between 45 and 60 days of pregnancy. When it is performed earlier in gestation, the pregnant uterus is still too small and too far away from the abdominal wall to be visualized. With progressing gestation, the amount of fluid in the uterus decreases relatively, which makes interpretation of the image more difficult and results in more false negative results when pregnancy detection is performed quickly.

In contrast to the determination of the ovPAG concentration, the outcome of the pregnancy detection by means of ultrasound is immediately available and ewes can simultaneously be separated into different groups. When blood is collected for determination of the ovPAG concentrations, it takes about 1 week before the results are available. On the other hand, when a blood sample is taken for determination of ovPAG concentration, it can also be used for other analyses, e.g. genotypical determination of scrapie resistance or serology (visna-maedi). This makes it possible to save money on blood collection.

Since late embryonic mortality can cause false positive results both with ultrasound and with ovPAG

determination, it is advisable to examine the ewes not earlier than D35. After 35 days of gestation, the number of fetal losses is normally very low in the absence of infectious abortion, and lambing rates can be predicted with a fairly high accuracy rate.

The number of ewes that can be examined for pregnancy by means of transrectal ultrasound or blood collection is similar (30-60/h), though a lot more can be examined when transabdominal ultrasound is performed (60-350/h). On small farms the cost for pregnancy diagnosis by means of ovPAG determination is equivalent to pregnancy diagnosis by means of transrectal ultrasound (3 euro / animal). However, when a sheep breeder is paying for pregnancy detection, he wants to have results with a high accuracy at every stage of gestation. This high accuracy can only be obtained by determination of the ovPAG concentration. The extra price paid for this detection method compared to ultrasound is negligible. Moreover, when determination of the ovPAG concentration is used for pregnancy detection, the results are registered and no discussion about the results obtained is possible. This is especially interesting when ewes are sold with a guarantee of being pregnant or in insurance issues.

On larger farms, however, it is more economical to use transabdominal ultrasound after 35 days of pregnancy due to the high number of ewes that can be examined per hour. The higher speed of detection with transabdominal ultrasound and the resulting lower price per ewe compensate for the lower accuracy compared to determination of the ovPAG concentration. For veterinarians who have no ultrasound, ovPAG determination remains a very good alternative for pregnancy diagnosis.

In conclusion, determination of ovPAG concentration by means of a homologous RIA is a more reliable test than transrectal ultrasound for pregnancy diagnosis in sheep at 25, 35 and 45 days of gestation. On large farms (>100 ewes), the higher speed of detection and the resulting lower price per ewe compensate for the lower accuracy compared to determination of the ovPAG concentration. As large sheep farms are very rare in Belgium, pregnancy diagnosis by homologous ovPAG RIA can be recommended for the majority of the country's sheep farms.

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