ASSESSMENT OF PROGESTERONE AND PREGNANCY-ASSOCIATED GLYCOPROTEIN CONCENTRATIONS FOR EARLY PREGNANCY DIAGNOSIS IN EWE

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Summary

Low fertility represents one of the causes for the decrease of production efficiency in animals, as well as the prolongation of the interval for service period (the interval between parturition and conception). In this regard, an early and highly precise diagnosis represents the most useful method to monitor the reproductive performances in animals. The objectives of this study were to determine a value of serum progesterone (P₄) concentration, assessed using an enzymeimmunoassay and oPAG levels determined with a heterologous RIA, for the early distinction between pregnant and non-pregnant ewes. Adult, non-lactating Palas Merino ewes (n = 50) were synchronized during the breeding season with progestagens and gonadotrophins and mated with fertile rams. Plasma was collected on days 0 (estrus), 14 and 25 from mating. The physiological state of the females was monitored till parturition (returns to estrus over 3 estral cycles, abortions, kidding date and the number of products obtained). The results show that the sensitivity of the RIA-PAG test proved to be 60.52% on day 14 and much higher on day 25 (94.5%). This means that on day 25, of the pregnant ewes, 94.5% had concentrations higher than 1.5 ng/ml. the RIA-PAG specificity was 70% on day 25, which indicates the fact that 70% of the non-pregnant ewes displayed concentrations way below 1.5 ng/ml. Also, the predictable values of gestation and non-gestation were high on day 25 (83.33% and 88.88%, respectively). The test accuracy was 86.10%. The results obtained led to the conclusion that the quantitative evaluation of the proteins associated to gestation (PAG) by the RIA technique can constitute a sure method to establish early gestation in ewe. The high level of progesterone 5-7 days after mating can be interpreted by the fact that ovulation occurred and fecundation chances are real. The correlation between the progesterone concentration and the PAG concentration and then the correlation with a positive echography can confirm the gestation or non-gestation diagnosis.

Key words. Sheep, progesterone level, PAG, pregnancy

Introduction

The intensive sheep management and the wide spread application of the controlled breeding techniques, such as artificial insemination and out-of season breeding, increase the need for an accurate and practical test for early pregnancy diagnosis. The traditional methods such as non-return to estrus and abdominal ballottement are not satisfactory. In addition, laparotomy, laparoscopy, rosette inhibition test and vaginal biopsy are accurate techniques, however these methods are impractical under farm conditions (Goel and Agrawal, 1992; Gordon 1999). Recently, pregnancy-associated glycoproteins (PAG) have been isolated from domestic ruminant placentas (Zoli et al., 1991 and 1995; Garbayo et al., 1998) and radioimmunoassays have been developed for their determination in the maternal plasma (Zoli et al., 1992, Perényi et al., 2002) or in the milk (González et al., 2000). In cattle and goats, the pregnancy-associated glycoprotein radioimmunoassay (PAG-RIA) accurately diagnose early pregnancy (González et al., 1999).
In sheep, PAG1-ov was identified in the blood of pregnant ewes. This protein was isolated from the placenta; the highest concentration is in the blood of ewes 2 months after mating and remains high till parturition.

The purpose of the research is to identify the pregnancy-associated glycoproteins in an early stage of gestation and to correlate their concentration with that of plasmatic progesterone.

Material and methods
Fifty Merino ewes (1.6-to -3-year-old) were used in the Study. The ewes were housed and managed at a ANCC Caprirom farm. In all ewes estrus was synchronized by insertion of intravaginal sponges containing 30 mg flurogestone acetate. All ewes were inseminated with fresh semen at 48 h after sponge removal. The day of insemination was considered as Day 0 for calculating the gestational period.

The plasma originated from 50 Merinos of Palas sheep and was collected on days 0 (estrus), 14 and 25 from mating. Blood samples (5 mL) were withdrawn from the jugular vein into heparinized vacutainer tubes, which were put into a cool box until centrifugation. The plasma was separated within 1 hours after collection by centrifugation at 1500 x g for 20 min, and then stored at -40°C until assayed for progesterone and PAG concentrations.

The physiological state of the females was monitored till parturition (returns to estrus over the period of 3 estral cycles, abortions, dates of lambing and the number of products obtained). The plasma samples were analyzed by RIA with the purpose of determining PAG and also by the ELISA technique in order to analyze the progesterone.

RIA analysis for the determination of PAG was accomplished in the Reproduction Physiology Laboratory – The Faculty of Veterinary Medicine (Liege, Belgium). The radioimmunological dosing was accomplished by RIA with pre-incubation for all the serums resulted from day 0 till day 25 from mating. The marked antigen and the antiseraums were offered by the Biotechnologies Laboratory in the Faculty of Veterinary Medicine (Liege, Belgium). The radioactivity of the immune complexes was measured on a Multi GammaCounter device, type WALACE.

ELIZA analysis for the determination of plasmatic progesterone. The determination of the serum progesterone was accomplished using the Progesteron DRG immunoenzymatic kit (Germany), which provides the material support for the quantitative determination of progesterone in serum and plasma, for the in vitro diagnosis. The analysis was accomplished using the Tecan device with annexes for reading, shaking, washing and the computerized processing of the data, owned by the Laboratory of Cellular and Molecular Biology in “Ovidius” University.

The test of the ELIZA Progesteron DRG Kit is based on the principle of competitive bonding. The determination plates display polyclonal antibodies for the antigenic situs of the progesterone molecule. The endogen progesterone in different samples from females is conjugated with the mare peroxidase in order to bind to the antibodies. After incubation, the unbounded conjugate is removed by washing. The quantity of conjugated peroxidase is inversely proportional to the concentration of progesterone in the sample. By adding substrate solution, the color intensity increases inversely proportional to the concentration of progesterone in the patient sample.

Results
Ovine pregnancy-associated glycoproteins (ovPAGs) are synthesized by binucleate cells of trophoblast, and belong to aspartic proteinase family (Xie et al., 1991) and most of them are without enzyme activity (Xie et al., 1997). They
have molecular weights between 43 to 67 kDa (Zoli et al., 1995, Xie et al., 1997). Due to the fact that the proteins are synthesized by the binucleate cells, with trophoblastic origin, which begin to migrate towards the endometrium at the moment of attaching to it, the PAG level during the first 14 days is low but detectable. The profile of PAG level increase in the pregnant females becomes obvious starting with day 21 of pregnancy. This is why the lambing results were used in order to verify the accuracy, sensitivity and specificity of the test.

Due to the detectable PAG levels in non pregnant females (other sources than the trophectoderm or the secretion of proteins similar to PAG), the ewes were considered pregnant at a plasmatic PAG concentration of 1.5 ng/ml.

As it can be observed, the medium level of gestation proteins on day 0, with an average value of 0.61±0.65 ng/ml, increases till day 14 (1.75±1.25) reaching an average value of 6.48±5.25 ng/ml (table 1) on day 25. The ewes were diagnosed as non-pregnant because of the PAG medium level and lack of lambing. In non pregnant ewes, there is a medium sub-unitary concentration (under the detection limit) of PAG (table 2).

<table>
<thead>
<tr>
<th>Lotto</th>
<th>Day</th>
<th>n</th>
<th>x±sx</th>
<th>Min - Max (ng/ml)</th>
<th>Mediana</th>
<th>Dev Std</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>23</td>
<td>1.56±0.15</td>
<td>0.49-2.9</td>
<td>1.66</td>
<td>1.12</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>23</td>
<td>12.69±1.43</td>
<td>4.65-33.99</td>
<td>12.60</td>
<td>46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>23</td>
<td>14.4±1.52</td>
<td>4.82-35.83</td>
<td>15.36</td>
<td>45</td>
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</tbody>
</table>

Conclusions

Early detection of pregnancy and determination of the fetal numbers have economical benefits to sheep producers. The method used for pregnancy diagnosis should be simple, accurate, rapid, inexpensive, practical and safe for both operators and animals. The sensitivity of the RIA-PAG test proved to be 60.52% on day 14 and much higher on day 25 (94.5%). This means that on day 25, of the pregnant ewes, 94.5% had concentrations above 1.5 ng/ml. The RIA-PAG specificity was 70% on day 25, which means that 70% of the non pregnant ewes displayed concentrations much under 1.5 ng/ml. Also, the predictable values of pregnancy and non pregnancy were high on day 25, namely 83.33% and 88.88%, respectively. The test accuracy was 86.10%.

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References