Early pregnancy diagnosis in sheep by progesterone and pregnancy-associated glycoprotein tests

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Introduction

Early pregnancy diagnosis is a useful management tool for sheep farming. Assessment of progesterone (P4) at Days 16 to 18 after AI is recommended as early pregnancy test with high sensitivity (100%)⁵. However, the specificity of the test for non-pregnancy was often shown to be relatively low (72%)⁶, because plasma P4 level is an indicator of a functional corpus luteum which can be associated with both pregnancy status or with ovarian or uterine pathologies like pseudopregnancy.

Ovine pregnancy-associated glycoproteins (ovPAGs) are secreted by the trophoblastic binucleate cells⁷, so they are good indicators for both pregnancy and feto-placental well-being⁸. By using a heterologous radioimmunoassay, ovPAG-1 can be detected in the blood of some pregnant ewes at week 3 after AI⁹. The concentration of ovPAG-1 varies according to the stage of gestation, the breed of the ewe and the number of fetuses⁹,¹⁰.

Aims

The aim of this study was to evaluate the accuracy of the heterologous PAG-RIA for the determination of early pregnancy in sheep and to compare the accuracy of the P4 and PAG tests on the pregnancy diagnosis in this species.

Materials and Methods

1) Animals and samples

- Estrus was synchronized in 182 Awassi x Merino ewes and blood samples (5 ml) were collected at Days 0 (Day of the insemination), 16 and 22 after artificial insemination (AI).

2) Radioimmunoassays

- Plasma P4 concentrations at Days 0 and 18 were determined by radioimmunoassay after an extraction step¹⁰.
- PAG concentrations at Day 22 were detected by a heterologous RIA using bovine PAG 67 kDa as tracer and standard, and rabbit antiserum raised against caprine PAGs 55 and 59 kDa (R798) as the first antibody¹¹.
- The cut-off value for both P4 and PAG assays to diagnose pregnant ewes was ≥ 1 ng/ ml.

3) Statistical analysis

- Data for both assays were arranged as follows: correct positive diagnosis (a), incorrect positive diagnosis (b), correct negative diagnosis (c), incorrect negative diagnosis (d). From these data, the sensitivity (100 x a/a+d), the specificity (100 x c/c+b), the positive predictive value (100 x a/a+b) and the negative predictive value (100 x c/c+d) of both tests were calculated.

Results

The accuracy of the P4 and PAG tests for diagnosing pregnancy are shown in Table 1. For diagnosis of non-pregnant ewes, PAG test had a significantly higher specificity then the P4 test (P<0.01).

<table>
<thead>
<tr>
<th>Days of pregnancy</th>
<th>No. of pregnant ewes</th>
<th>Pregnancy test</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>Sensitivity (%)</th>
<th>Specificty (%)</th>
<th>Predictive Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>182</td>
<td>P4</td>
<td>31</td>
<td>7</td>
<td>144</td>
<td>0</td>
<td>100</td>
<td>954</td>
<td>81.5</td>
</tr>
<tr>
<td>22</td>
<td>182</td>
<td>PAG</td>
<td>29</td>
<td>0</td>
<td>151</td>
<td>2</td>
<td>95.5</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

α: correct positive diagnosis (pregnant), b: incorrect positive diagnosis (non-pregnant), c: correct negative diagnosis (non-pregnant), d: incorrect negative diagnosis (pregnant). Asterisk indicates significant differences between P4 and P4 test (P<0.01).

The P4 and ovPAG concentrations for both pregnant and non-pregnant ewes are shown in Table 2. There was a significant difference between the pregnant and non-pregnant levels on both P4 and PAG concentrations (P<0.0001).

<table>
<thead>
<tr>
<th>Days of pregnancy</th>
<th>P4</th>
<th>Non-pregnant ewes</th>
<th>PAG</th>
<th>Non-pregnant ewes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>Concentration (ng/ml)</td>
<td>151</td>
<td>0.2±0.1²</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td></td>
<td>121</td>
<td>0.4±0.1²</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td></td>
<td>151</td>
<td>0.2±0.1²</td>
</tr>
</tbody>
</table>

²: Different superscripts in the same line indicate a significant (P<0.0001) difference between pregnant and non-pregnant ewes.

Conclusions

In conclusion, the PAG-RIA test is a reliable method for diagnosing pregnancy status as early as 22 days after AI. The PAG test has advantages over P4 test: it can be used at any period of pregnancy after day 22 and it can differentiate between pregnancy and prolonged inestrum interval.

References


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