

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

A. Karen¹, J.F. Beckers², J. Sulon², N.M. Sousa², K. Szabados³, J. Reczigel⁴ and O. Szenci¹

¹Department of Large Animal Clinical Sciences, Faculty of Veterinary Science, H-1400 PO Box 2, Budapest, Hungary.

²Department of Physiology of Reproduction, Faculty of Veterinary Medicine, University of Liège, B-4000, Belgium.

³Awassi Corporation, Bakonszeg, Hungary.

⁴Biomathematics and Informatics Department, Faculty of Veterinary Medicine, Budapest, Hungary.

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%)⁽¹⁾. However, the specificity of the test for non-pregnancy was often shown to be relatively low (72%)⁽¹⁾, 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 cells⁽²⁾, so they are good indicators for both pregnancy and feto-placental well being⁽³⁾. By using a heterologous radioimmunoassay, ovPAG-1 can be detected in the blood of some pregnant ewes at week 3 after AI⁽⁴⁾. The concentration of ovPAG-1 varies according to the stage of gestation, the breed of the ewe and the number of fetuses⁽³⁻⁵⁾.

Aims

The aims of this study were 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), 18 and 22 after artificial insemination (AI).

2) Radioimmunoassays

- Plasma P4 concentrations at Days 0 and 18 were determined by radioimmunoassay after a extraction step⁽⁶⁾.
- 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 (R708) as the first antibody⁽⁷⁾.
- 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 ($100 \times a/a+d$), the specificity ($100 \times c/c+b$), the positive predictive value ($100 \times a/a+b$) and the negative predictive value ($100 \times c/c+d$) of both tests were calculated.

This work was supported by Hungarian Research Fund, Belgium Ministry of Agriculture and FNRS/Belgium.

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 than the P4 test ($P < 0.01$).

Table 1: Sensitivity, specificity and predictive values of the progesterone (P4) and pregnancy-associated glycoprotein (PAG) tests in Awassi x Merino ewes.

Days of pregnancy	No. of ewes	Pregnancy test	a	b	c	d	Sensit. (%)	Specif. (%)	Predictive Value (%)	
									Positive	Negative
18	182	P4	31	7	144	0	100	95.4	81.5	100
22	182	PAG	29	0	151	2	93.5	100*	100	98.7

a: 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 PAG 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 2: Concentrations (mean \pm SD) of P4 and ovPAG in pregnant and non-pregnant ewes.

Days of pregnancy	RIA	Non-pregnant ewes		Pregnant ewes	
		n	Concentration (ng/ml)	n	Concentration (ng/ml)
0	P4	151	0.2 ± 0.3^a	31	0.2 ± 0.1^a
18	P4	151	0.4 ± 0.4^a	31	3.3 ± 0.9^b
22	ovPAG	151	0.2 ± 0.1^a	31	4.3 ± 1.4^b

^{a,b} Different superscripts in the same line indicate a significant ($P < 0.0001$) difference between pregnant and non-pregnant ewes.



Merino



Awassi

Conclusion

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 inter-estrus interval.

References

- (1) Susmel P and Piasentier E. *Small Rum. Res.* 1992, 8:325-332.
- (2) Green JA et al. *Biol Reprod* 2000, 62:1624-1631.
- (3) Wallace JM et al. *J. Reprod. Fert.* 1997, 109:53-58.
- (4) Gajewski Z et al. *Adv. Cell Biol.* 1999, 26 (suppl.12): 89-96.
- (5) Ranilla MJ et al. *Theriogenology* 1994, 42: 537-546.
- (6) Ranilla MJ et al. *Small Rum. Res.* 1997, 24:125-131.
- (7) Gonzalez et al. *Theriogenology* 1999, 62:717-725.