

EARLY PREGNANCY DIAGNOSIS IN EWES

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Estrus was synchronized in 22 ewes of different breeds using the Veramix system during the anoestrous season and early pregnancy diagnosis was made on the basis of high plasma progesterone concentration at 16/18 and 21 days after mating. The general accuracy, sensitivity and specificity of the radioimmunoassay of plasma progesterone for early pregnancy diagnosis were 81.82, 88.22 and 60%, respectively, and diagnosis of pregnancy was confirmed by real-time ultrasound scanning at 90 or 120 days after mating or lambing results. Ultrasound scanning was 100% effective in the pregnancy diagnosis et the examined ewes.

Key words: Estrus, plasma pregesberone, pregnancy, ewes

INTRODUCTION

Early pregnancy diagnosis is essential for successful reproduction in ewes. The development of radioimmunoassay techniques (RIA) for the assay of hormones has provided now possibilities for a more precise study of the endocrinological aspects of infertility. Progesterone levels in body fluids (milk, blood serum or plasma) are closely related to the growth and secretory function of the corpus luteum in the normal cycle and in early pregnancy (Đurđević et al., 1982). Plasma progesterone level in pregnant ewes must increase during the period from Day 3. to Day 7. after mating to the values typical of those observed during the luteal phase and remain high until placental secretion of progesterone is well established (Ashworth et al., 1989; Miller & Moore, 1976; Wilmut et al., 1985). Therefore we decided to estimate general accuracy, sensitivity and specificity of RIA of plasma progesterone for early pregnancy diagnosis in ewes.

MATERIAL AND METHODS

Estrus was synchronized in 22 ewes of different breeds during the aneestrous season using the Veramix system (12 days of 60 mg of MAP followed by 700 IU of PMSG i/m at the removal of pessaries), and all ewes were introduced to the fertile rams 48 hours after the removal of pessaries. Plasma progesterone concentration was determined at 16/18, 21. and 90. or 120 days after mating.

Pregnancy diagnosis was confirmed by real-time ultrasound scanning at 90. or 120. days after mating or by the lambing results.

Plasma progesterone concentration was determined using a commercial kit (INEP-Zemun) and Gammachem 4800 scintillation counter. Ewes with a plasma progesterone level more than 1.59 nmol/l at 16/18 or 21 days after mating were classified as pregnant (Robertson & Sarda, 1971; Thimonier et al., 1977; Langford, 1982).

Estimation of the usefulness of RIA of plasma progesterone in early pregnancy diagnosis was made by calculating general accuracy, sensitivity and specificity:

$$\begin{aligned} \text{General accuracy} &= \frac{\text{number of correct diagnoses}}{\text{number of ewes}} \times 100 (\%) \\ \text{Sensitivity} &= \frac{\text{pregnant ewes classified as pregnant by RIA}}{\text{pregnant ewes}} \times 100 (\%) \\ \text{Specificity} &= \frac{\text{nonpregnant ewes classified as nonpregnant by RIA}}{\text{nonpregnant ewes}} \times 100 (\%) \end{aligned}$$

RESULTS AND DISCUSSION

Plasma progesterone concentrations and the results of lambing and ultrasound scanning are given in Table 1.

Our results in early pregnancy diagnosis using plasma progesterone concentration are based on the pattern of plasma progesterone during the estrous cycle and early pregnancy in ewes. Studies in ovariectomized ewes have revealed three important phases in progesterone secretion after mating (Miller & Moore, 1976; Wilmut et al., 1985). First, it is essential to have a low concentration of progesterone (probably 0.38-0.76 nmol/L) from the first day of the estrous cycle. Second, the concentration must increase during the period from 3-7 days after mating to values typical of those observed during the luteal phase (9.54-19.08 nmol/L). Third, it is important that this luteal phase value is adequate for the maintenance of pregnancy.

The general accuracy, sensitivity and specificity of RIA of plasma progesterone for early pregnancy diagnosis were 81.82, 88.22 and 60%, respectively. These results are in accordance with those reported by other authors (Popovski et al., 1991; Đurđević et al., 1982). There is general agreement that the incidence of false positive diagnoses is related to embryonic death, uterine and ovarian pathology. In our study a false positive diagnosis was found in ewes No. 14 and 20. These ewes had high plasma progesterone concentrations at 16/18 days after mating (3.05 and 6.05 nmol/L) but ewe No. 14. at 90. days after mating had only 0.48 nmol/L, while in ewe No. 20 plasma progesterone concentration declined to 1.81 nmol/L at 21. days after mating. These data indicate that the false positive diagnosis is based on embryonal death in those ewes.

However, in our opinion the expressions "false diagnosis" or "error of the method" are not quite correct because our results and those of other authors (Đurđević et al., 1982) indicate that the animals which were examined in the

early days pregnancy really were pregnant. Their embryos did not survive and it was not possible to confirm the positive diagnosis of pregnancy in the later period of gestation, but it does not necessarily mean that early pregnancy diagnosis based on plasma progesterone concentration was incorrect.

Table 1. Plasma progesterone concentration (nmol/L), results of lambing and ultrasound scanning in ewes.

number of ewe	days after mating			pregnancy diagnosis		
	16/18	21	90/120	RIA	ultrasound scanning	lambing
1.	10.40	8.4	—	P	+	+
2.	10.40	3.55	45.70	P	+	+
3.	12.40	—	89.10	P	+	+
4.	7.48	3.46	2860	P	+	+
5.	15.90	7.95	38.10	P	+	+
6.	4.38	1.71	15.40	P	+	+
7.	7.49	11.76	47.40	P	+	+
8.	10.90	—	10.60	P	+	+
9.	69.90	—	36.90	P	+	+
10.	68.20	—	71.10	P	+	+
11.	20.90	—	75.60	P	+	+
12.	1.41	—	74.70	non-P	+	+
13.	0.59	—	16.70	non-P	+	+
14.	3.05	—	0.48	P	—	—
15.	0.50	—	0.49	non-P	—	—
16.	0.44	—	0.67	non-P	—	—
17.	0.39	—	0.43	non-P	—	—
18.	2.40	9.49	—	P	+	+
19.	5.22	3.93	—	P	+	+
20.	6.05	1.81	—	P	+	+
21.	5.15	2.32	—	P	+	+
22.	7.64	8.45	—	P	+	+

In ewes No. 12. and 13. plasma progesterone concentration at 18. day after mating was very low but later analysis of plasma progesterone showed that those ewes were pregnant. These results were confirmed by ultrasound scanning and lambing results. However, these ewes lambed almost 30 days later than the others. This fact indicated that they did not conceive at the time of synchronized estrus, but later.

A very high plasma progesterone concentration was found in ewes No. 9. and 10. These results indicate that those ewes had accessory corpora lutea which secreted an additional amount of progesterone (Novoa, 1986; Dobeli & Schwander, 1983; Ashworth et al., 1989). This high plasma progesterone concentration could be also partly explained by the breed difference (Novoa, 1986).

Popovski et al., (1989) reported that early pregnancy diagnosis in ewes can be made at 18, days after mating, but plasma progesterone concentration in pregnant ewes was relatively high (4.36 ± 0.57 nmol/L). Our results indicate that ewes with a plasma progesterone concentration higher than 1.59 nmol/L at 16/18 days after mating can be considered as pregnant.

Real-time ultrasound scanning can be effectively used for pregnancy diagnosis from 40-45 days of gestation onward (Popovski et al., 1991; Blasco et al., 1989). It can be also used for the prediction of ovine fetal age (Sergeev et al., 1990). Our results showed that ultrasound diagnosis of pregnancy at 90. or 120. days after mating is 100% effective.

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RANA DIJAGNOSTIKA GRAVIDITETA KOD OVACA

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SADRŽAJ

Posle sinhronizacije estrusa kod 22 ovce različitih rasa primenom Veramix sistema izvršena je rana dijagnostika graviditeta određivanjem koncentracije progesterona 16/18 i 21. dana od parenja. Opšta tačnost progesteronskog testa u ranoj dijagnostici graviditeta bila je 81.82%, senzitivnost 88.22% i specifičnost 60%, a dijagnoza je potvrđena ehografijom 90. ili 120. dana od parenja i rezultatima jagnjenja. Ultrazvučna dijagnoza graviditeta je 100% efikasna u ispitivanom periodu.