STUDIES ON THE EARLY PREGNANCY DETERMINATION IN COWS BY USING THE ENZYME-IMMUNOASSAY AND RADIO-IMMUNOASSAY IN MILK

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Summary

Milk samples(n = 78) were taken 19d, 20d, 21d, 22d after artificial insemination(AI) for early pregnancy diagnosis by using the Enzyme immunoassay(EIA) kit. The progesterone (P_4) concentration in the whole milk was measured on the same day of pregnancy diagnosis. Rectal palpation(RP) was accomplished between 60d and 70d after AI to estimate the ovary condition and pregnancy status.

Milk progesterone concentrations measured by Radio-immunoassay(RIA) method, in the pregnant cows at 17d, 19d, 21d after insemination were 17.10 ± 0.91, 17.60 ± 0.46, and 18.43 ± 0.79 nmol/l, whereas those in the not-pregnant cows were 6.57 ± 1.03, 2.63 ± 0.29, and 0.67 ± 0.08 nmol/l, respectively. When the progesterone concentration was less than 7 nmol/l, the color of the EIA kit was lighter and when the progesterone concentration was ≥ 16 nmol/l, the color of the EIA kit was darker compared to the standard color. The detection rates of error by judging the color differences were 5.1% and 20.7%, respectively. In the early pregnancy diagnosis by the EIA kit and RIA method, the accuracy rates in the pregnancy of cows were 82% and 87%, and those in not-pregnant cows were 86% and 91%, respectively. For ovarian status estimated by the RIA method and certified by RP, the accuracy rates of the ovarian atrophy, follicular cyst and luteal cyst were 80, 91 and 83%, and the progesterone concentrations were 2.51, 2.03, and 26.7 nmol/l, respectively.

(**Key Words** : Milk Progesterone, Pregnancy Diagnosis, Ovarian Status, EIA, RIA)

Introduction

In order to achieve the economically optimal calving interval in dairy cattle it is essential to reduce the length of days open to a minimum, since the time interval between calving and subsequent conception is the main objective which could be manipulated.

It has been well known that progesterone concentrations in blood and milk are closely related to reproductive stages. Progesterone concentration is lowest 2 to 3d before estrus and remains at low level until 4 to 5d after ovulation during formation of the corpus luteum (CL) (Van de Wiel et al., 1978; Foote et al., 1979; Chang and Estergreen, 1983).

When a cow is not inseminated or fails to conceive following insemination, the CL regresses and progesterone level in blood and milk declines prior to estrus. In contrast, when the insemination is followed by conception, CL is maintained, the cow does not return to estrus, and progesterone concentration remains elevated throughout pregnancy.

As an indicator of the cow's reproductive status, progesterone plays an important role. The following management uses have been suggested for the test. 1) early pregnancy detection, 2) confirmation of estrus at time of breeding, 3) determination of the resumption of ovarian activity after calving, and 4) design of specific treatments for various cystic and other pathological conditions associated with ovarian activity (Nebel, 1985; Fagan and Roche, 1986).

Milk progesterone tests can be used to detect early pregnancy in cows after service or to prevent insemination errors. Accurate determination of cows which are pregnant, and more importantly, cows which are not, is an essential part of good reproductive management. As a method of pregnancy diagnosis, milk progesterone concentration at 21 to 24 d postinsemination has 67 to 88% of accuracy for diagnosing cows subsequently determined to be pregnant and 91 to 100% of accuracy for cows determined to be not-pregnant (Heap et al., 1976; MacFarlane et al., 1980; Wishart et al., 1975; Pope et al.,

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1976; Pennington et al., 1985). The objectives of this study are 1) to develop the method for the determination of early pregnancy in dairy cattle 2) to establish the diagnosis system from ovarian status, therefore, 3) to shorten the calving intervals.

Materials and Methods

EIA

From 78 Holstein dairy cows whole milk samples (50ml) were collected at the end of milking in tubes and samples were analyzed by EIA kit (Farmos diagnostica, Finland) (figure 1).

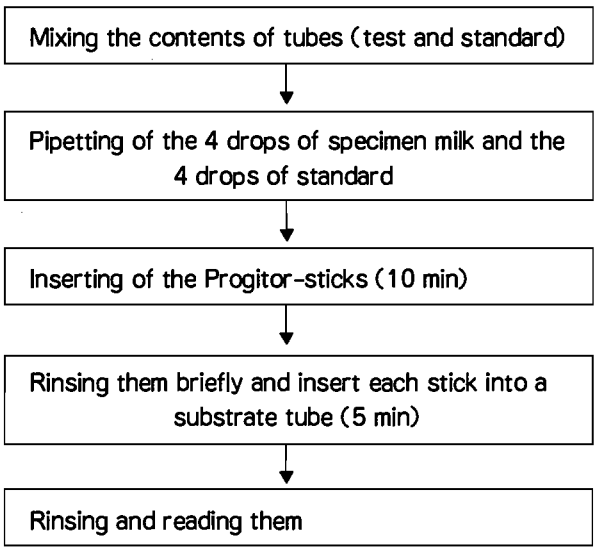

Figure 1. Flow chart of assay procedure by EIA kit

RIA

From 78 Holstein dairy cows whole milk samples (50 ml) were collected at the end of milking in tubes or bottles containing a sodium azide tablet as preservative. Samples were stored at 2-8℃ until analysed for progesterone. Concentration of progesterone was assayed by using the progesterone[^{125}I] kit (Farmos diagnostica, Finland) (figure 2).

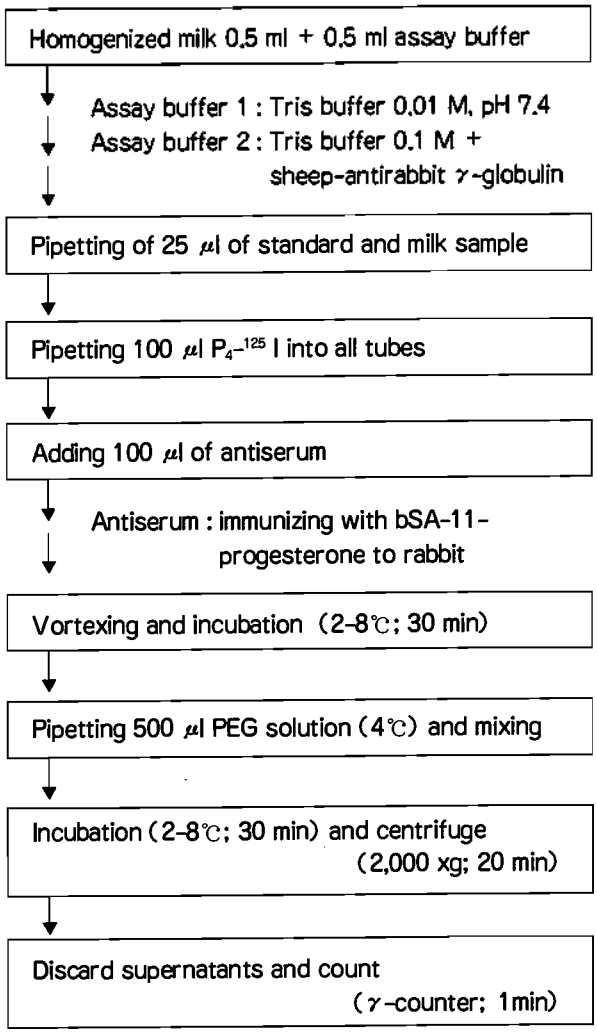

Figure 2. Flow chart of assay procedure by RIA method

Results

The logit-log standard curve of concentration of progesterone and linear regression equation (Y = 2.740 − 2.189 X; r = 0.98) are shown in figure 3.

In this study, for the cows diagnosed by RIA method as pregnant and not-pregnant 19d, 21d, and 23d after artificial insemination were 73.7 (28/38) vs 91.9% (34/37), 81.6 (31/38) vs 91.9% (34/37), and 84.2 (32/38) vs 100%

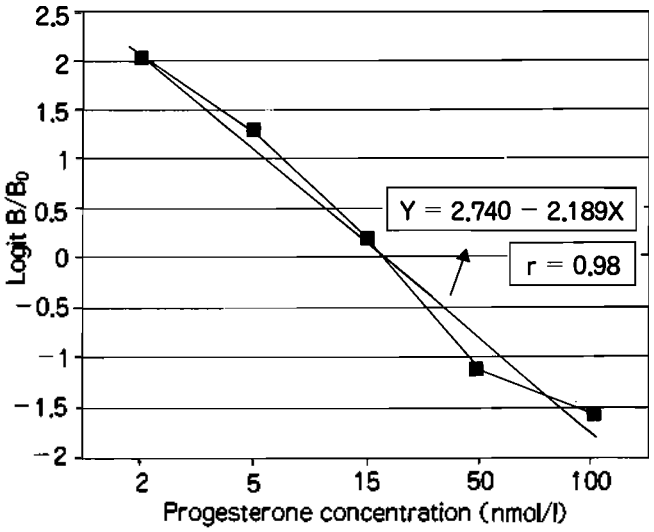

Figure 3. Logit-log standard curve of progesterone concentration nmol / l

(37/37), respectively. At that time, progesterone concentrations were 15.0 vs 3.3 nmol/l, 15.9 vs 2.1 nmol/l, and 16.7 vs 1.5 nmol/l, respectively (table 1).

As progesterone concentration was ≤ 7 nmol/l, color change lighter than EIA kit standards was 5.1% (2/39), and as progesterone concentration was ≥ 16 nmol/l, color change darker than EIA kit standards was 20.7% (6/29). And, total error percentage in the EIA kit was the 11.7% (8/68) (table 2).

Accuracy rates for pregnant cows by EIA kit and RIA method were 82 and 86%, and for not-pregnant cows were 87 and 91%, respectively (table 3). Relative accuracy rates of EIA kit compared to RIA method was 95.3% in the pregnant cows and 95.6% in the open cows. Progesterone concentration of the pregnant and not-pregnant cows was the 25.7 and 1.81 nmol/l, respectively.

As shown in table 4. accuracy rates for ovarian atrophy, follicular cyst, and luteal cyst diagnosed by EIA kit and certified by RP were 80, 91 and 83%, respectively. At that time, progesterone concentration of each ovarian status was 2.51, 2.03 and 26.7 nmol/l, respectively.

TABLE 1. ACCURACY OF EARLY PREGNANCY DIAGNOSIS DETERMINED BY P_4 LEVEL BY RIA METHOD

	19 d		21 d		23 d	
	P_4 level (nmol/l)	Accuracy (%)	P_4 level (nmol/l)	Accuracy (%)	P_4 level (nmol/l)	Accuracy (%)
Pregnant	15.0	73.7 (28/38)	15.9	81.6 (31/38)	16.7	84.2 (32/38)
Not pregnant	3.3	91.9 (34/37)	2.1	91.9 (34/37)	1.5	100 (37/37)

TABLE 2. COLOR CHANGE BY PROGESTERONE CONCENTRATION USING EIA KIT

Progesterone concentration	Color change				Total
	light	standard	medium	dark	
≤ 7.0 nmol/l	2 (5.1)		13 (33.3)	24 (61.5)	39
8 – 15	4 (40.0)		5 (50.0)	1 (10.0)	10
16 ≥	23 (79.3)		4 (13.8)	2 (6.9)	29

( )value indicates the percentage.

TABLE 3. ACCURACY RATES OF EIA KIT AND RIA METHOD FOR DIAGNOSIS OF PREGNANCY

Classification	No. of cows	EIA(A)	RIA(B)	A/B
Pregnant	22	82%	86%	95.3%
Not pregnant	23	87	91	95.6

TABLE 4. DIAGNOSIS OF OVARIAN STATUS BY RIA, EIA AND CERTIFIED BY RP

Cow/Ovarian status	Ovarian atrophy	Follicular cyst	Luteal cyst	Normal cow	
				Pregnant	Not pregnant
No. of cows	10	11	12	22	23
Certified No.	8	10	10	18	20
Accuracy (%)	80	91	83	82	87
Progesterone conc. (nmol/l)	2.51	2.03	26.7	–	–

Discussion

The overall accuracy rates of EIA kits in predicting pregnancy and ovarian status appeared to relatively lower to those of RIA kit and RP. But this result is consistent with other reported laboratory diagnosis accuracies of 67 to 88% for pregnant and 87 to 100% for not-pregnant cows (Heap et al., 1976; MacFarlane et al., 1977; Pennington et al., 1976, 1985; Pope et al., 1976; Booth and Holdsworth, 1976; Nebel et al., 1987; Nebel, 1988).

And overall error detection rate for diagnosis of the pregnancy was (20.7%), as progesterone concentration was

≥ 16 nmol/l color change more than EIA kit standards, was relatively higher to those of the not-pregnant (5.1%), as progesterone concentration was ≤ 7 nmol/l color change less than EIA kit standards. That result in the pregnancy diagnosis was slightly higher than that of EIA kit standard.

Ovarian cysts (follicular cyst and luteal cyst) is one of the major infertility problems in dairy cattle. Cystic cows having a low progesterone concentration are considered to have follicular cyst and those with high concentration are classified as having luteal cyst. Accuracy diagnosis rate of follicular cyst (91%) by the EIA kit was relatively higher than that of ovarian atrophy (80%), or luteal cyst (83%), respectively. However, the accuracy with which on-farm progesterone test was used or the proportion of follicular relative to luteal cysts did not significantly affect (Ruiz et al., 1992).

Time of insemination, early pregnancy and ovarian status diagnosis were predicted by EIA kits with an accuracy rate comparable to that of the RIA and rectal palpation (Wimpy et al., 1986; Romagnolo et al., 1993). However, result obtained in study suggest that EIA kit had a significantly higher accuracy rate in the not-pregnant cows than that of pregnant cows. Conclusively, milk progesterone concentration, as measured by EIA kit progesterone tests, was a good predictor of the reproductive status of the cows and this technique will contributed to increase of the farmer's income.

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