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PROGESTERONE ASSAY FOR MONITORING POSTPARTAL OVARIAN ACTIVITY AND EARLY PRECNANCY DIAGNOSIS IN DAIRY COWS

(With 2 Tables & 1 Figs.)

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تحليل هرمون البروجستيرون لتعيين نشاط المبيض بعد الولاده وكذلك تشخيص الحمل المبكر في الأبقـــار

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تم تقييم نشاط المبيض في الأبقار بعد الولادة وذلك بقياس هرمون البروجستيرون يبدأ نشـــاط المبيض في الأبقار الطبيعية فيما بين اليوم ٢٠ الى ٣٠ ولكن يتأخر لليوم ٤٠ في الأبقار غير الطبيعية. وتم تقييم طريقة التحليل بطرق الأنزيم المناعي لتعيين البروجستيرون ووجد أن هناك علاقة ارتباط عاليـــة (معامل الأرتباط ٠٫٨٦٦) بين هذه الطريقة وطرق الاشعاع المناعي . وقد أستخدمت هذه الطريقة لتشخيص الحمل المبكر (١٨ ـ ٢٥ يوم) بعد التلقيح حيث كانت نسبة نجاح هذه الطريقة في تشخيص الحمـــــل الايجابي ٩١٫٤٪ وفي الحمل السلبي ٧٩٫٣٪ . وأكدت هذه الطريقة أنها علمية واقتصادية وغير ضـــــارة بالصحة اذا أستخدمت لتعيين نشاط المبيض بعد الولادة وكذلك تشخيص الحمل المبكر.

SUMMARY

Postpartum ovarian activity was evaluated in dairy cows by measuring serum progesterone concentration. In normal Holstein cows, ovarian activity started between day 20 and 30, while it was delayed up to day 40 in abnormal cows.

A solid phase enzyme immunoassy for progesterone determination was developed and evaluated. The correlation coefficinet among serum progesterone concentration as determined by enzyme immunoassy and radioimmunoassy was high (r = 0.866). This technique was used to diagnose early pregnancy by measuring milk progesterone levels 18-25 days post insemination. The overall success rates of the positive or negative pregnancy test were 91.4% and 79.3%, respectively. This study confirms that enzyme immunoassay is a practical and economical method to monitor postpartum cyclicity and to diagnose early pregnancy in cattle. This method eliminates the hazard of using radioisotopes, especially in developing countries.

INTRODUCTION

Reproduction efficiency of dairy cattle is low (PELISSIER, 1972 and SPALDING, EVERETT and FOOTE, 1975). Many causes have been identified but anestrus, or failure to detect estrus, is a major factor in most studies. The economics of cattle breeding and milk production depend greatly on the interval from calving to conception. The average interval of clinical uterine involutin varies between 25 to 50 days (MOLLER, 1970 and BRITT, MORROW, KITTOK and SEGUIN, 1974). This interval influenced by many factors, such as environment, nutrition, age, different metabolic and puerperal disorders. Under physiological conditions the ovaries of some cows begin to function as early as 10-18 days after calving (MORROW, ROBERTS and McENTEE, 1969 and THATCHER and WILCOX, 1973).

Progesterone, a key hormone in regulating the estrus cycle, has been measured in blood and milk to provide information concerning the reproductive status of the animal (NANDA, TAKKAR and SHARMA, 1984, SCHIAVO, MATUSZCZAK, OLTENACU and FOOTE, 1975, SHEMESH, AYALON and MAZOR, 1979, and SINGH and PUTHIYANDY, 1980). Radioimmunoassay (RIA) has been the predominant analytical technique used to measure progesterone concentration. However, the RIA has several limitations, which are inherent to the use of radioactive isotopes. In recent years, enzyme immunoassay (EIA) methods for progesterone determination were developed by several investigators (MUNRO and STABENFELDT, 1984 and VAN DE WIEL and KOOPS, 1986). This assay was used primarily for early pregnancy diagnosis in several animal species (CHANG and ESTERGREEN, 1983 and CLEERE, GOSLING, MORRIS, CHARLETON, MOLONEY and FOTTRELL, 1985).

This study was undertaken to monitor the postpartum cyclicity in dairy cows by measuring progesterone concentration utilizing both RIA and EIA technique. In addition, the EIA technique for milk progesterone determination was evaluated in diagnosing early pregnancy.

MATERIAL and METHODS

Postpartum blood samples were collected starting at day one after calving. Samples were obtained twice a week from 10 Holstein cows up to day 70. All blood samples were obtained by jugular venipuncture. The samples were chilled in ice and transported to the laboratory. Blood samples were centrifuged at 3000 rpm for 20 minutes after a clot had formed. Serum samples were kept frozen at -20°C until analyzed for progesterone by RIA (progesterone RIA kits, Diagnostic Product Corp., Los Angeles, California, USA) and EIA. A microtiter plate EIA method was developed. Enzyme-labelled progesterone (E-P) was prepared by conjugating progesterone-3-O-carboxymethyloxime to horseradish peroxidase (Sigma Chemical Company) as previously

described (MUNRO and STABENFELDT, 1984). A specific progesterone antiserum was generously provided by Dr. James R. Harness (MELOY Laboratories, Virginia, USA). The cross reaction of this antiserum with other steroids was less than 3%. O-phenylenediamine 2 HCl was used as a chromogen and the assay procedures was exactly as described previously (MUNRO and STABENFELDT, 1984).

Because the individual profiles of the animals show phases of different length and progesterone peaks, it is difficult to compare them. Therefore, the area under the progesterone curve ($\int_0^t$ C progesterone Δ t) was integrated and plotted versus time (HARASZTI, HUSZENICZA, MOLNAR, SOLTI and CSERNUS, 1985).

Milk samples for pregnancy diagnosis were obtained from a total of 64 cows between day 18 and 25 post insemination. The total number of milk samples collected was 222. These samples were classified as samples taken for one day (single samples), samples taken from the same cow for two successive days (two samples) and samples taken from the same cow for three successive days (three samples). Following milking and thorough mixing of the milk, a sample was taken from the collection jar into a numbered universal container containing a Lactab mark 11 tablet (mercuric chloride and potassium dichromate). All samples were stored at 4°C until assayed for progesterone by EIA.

Data were statistically analyzed according to GILL and HAFS (1971) for analysis of repeated measurements.

RESULTS

Postpartum serum progesterone concentrations for individual Holstein cows are shown in Fig. 1. As early as 20 days postpartum, ovarian activity was evident in 2 cows (## 361 and 204). The progesterone profiles indicated two regular ovarian cycles with normal hormone levels within 60 days postpartum. Cow ## 352 showed 3 cycles within 70 days, however, the first cycle was of short duration (approximately 15 days). The first cycle for cow ## 349 was short with very low progesterone concentration, while the second cycle was typically normal.

Other cows exhibited different progesterone patterns. The cyclicity started after day 30 in 2 cows (## 340 and 146). Cow ## 340 showed 2 regular consecutive cycles, while cow ## 146 had a high progesterone level up to day 65.

The first increase in progesterone was detected after day 40 in 2 cows (## 362 and 299). Each cow exhibited a regular estrous cycle thereafter. The cyclicity of cow ## 211 was irregular until day 35 followed by a relatively long estrous cycle (approximately 28 days).

Cows were classified into normal or abnormal according to their integrated progesterone concentration. Table 1. Shows the total integrated area for progesterone levels. When the serum RIA or EIA area is below 30 or 50, respectively the animal is considered abnormal.

In normal cows, the area was significantly ($P < 0.05$) increased at day 28, while it increased in the abnormal cows at day 44 (EIA) and day 51 (RIA). A significant ($P < 0.05$) difference in the magnitude of the progesterone area between the normal and abnormal cows was detected at day 34 (RIA) and day 37 (EIA) (Table 1 and Fig. 2).

The relationship between the presence or absence of an active corpus luteum by rectal palpation and levels of higher or lower serum progesterone was 74% (14/19) and 67% (10/15), respectively. There was a significant correlation coefficient (r=0.866) between serum progesterone assays as determined by RIA and EIA (n = 136).

$$Y = 0.36 + 1.31 x$$

where Y = EIA (ng/ml)

and X = RIA (ng/ml)

Table 2 shows the pregnancy diagnosis results from milk progesterone by EIA. The success rates of the positive and negative pregnancy test in single samples ranged from 80 - 100% and 66 - 91%, respectively. The accuracy of the test was improved by the use of 2 samples (84 - 100% for pregnant) and (77 - 100% for nonpregnant). The accuracy of positive pregnancy diagnosis was 100% based on a single sample (day 25), 2 sample; (day 24 and 25) and 3 samples (day 23, 24 and 25). The overall success rate for the EIA method was 91.4% and 79.3% for positive and negative pregnancy, respectively.

DISCUSSION

There was considerable variation in the progesterone profiles during the postpartum period among the 10 cows studied (Fig. 1). The calculation of the progesterone dynamics differentiated between the normal and abnormal cows. Based on the total progesterone integrated area, 3 cows out of 10 were classified as abnormal (Cows 299, 362 and 211). These 3 cows did not show an increase in progesterone levels up to day 40 after parturition.

Postpartum pathological conditions such as metritis and retained placenta are the primary factors for reproductive disorders. Clinical examination revealed that the 3 abnormal cows had severe metritis associated with vaginal purulent discharge. Subclinical metabolic disorders such as ketosis and fatty liver have been shown to decrease the progesterone area in postpartum dairy cows (HARASZTI, et al. 1985).

The ovarian activity started between day 20 and 30 in the normal cows, while it started after day 40 in the abnormal cows. Under physiological conditions the ovaries of some cows begin to function as early as 10 - 18 days postpartum (MORROW, et al. 1969 and THATCHER and WILCOX, 1973).

The EIA technique for progesterone measurement proved to be of clinical value for routine monitoring of ovarian activity and pregnancy diagnosis in dairy cows. There was a good correlation between RIA and EIA for measurement of serum progesterone. However, the EIA values were higher than the RIA levels. The reason for this finding is not clear but the presence of interfering substances in plasma of some cows in pasture could be involved (SMITH, VAN RAVENSWAAY and McDONALD, 1986).

The EIA method showed that measurement of progesterone directly (ie. without extraction) in milk is reliable, simple and quick for pregnancy diagnosis. The method can be used in both highly automated laboratories with a large throughout of samples, and in simple field laboratories with no facilities for centrifugation or spectrophotometry. The use of microtiter plates selected as a solid phase antibody carrier eliminates the centrifugation and error involved when the tube content is poured with possible loss of some antibody.

In conclusion, the use of this EIA would allow pregnancy diagnosis as early as 18 - 25 days after insemination. This enables producers to proceed to early rebreeding and subsequently reducing the calving intervals.

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Table (1)
Integrated progesteronearea (ng/ml) during a 65 days postpartum for 7 normal and 3 abnormal cows

Days postpartum	Area of normal cows		Area of abnormal cows	
	RIA SE	EIA SE	RIA SE	EIA SE
3	1.32 (0.24)	1.65(0.19)	0.85(.38)	2.32(.90)
6	1.76(.30)	2.57(.16)	1.18(.46)	3.23(1.23)
9	2.36(.37)	3.90(.26)	1.93(.85)	4.80(1.66)
13	2.65(.36)	4.59(.32)	2.63(.71)	5.37(1.67)
16	3.48(.44)	6.63(.77)	2.97(.70)	6.81(1.58)
20	4.60(.93)	7.91(.79)	3.12(.70)	7.39(1.59)
25	7.95(2.31)	12.0(1.10)	3.72(.80)	8.61(1.61)
28	10.59(3.12)*	16.49(2.48)*	4.03(.80)	9.45(1.55)
31	17.07(4.22)	22.94(3.09)	4.92(1.05)	10.93(1.57)
34	21.45(5.03)[a]	30.29(5.23)	5.36(1.24)[b]	11.71(1.61)
37	29.45(4.79)	45.77(5.93)[c]	5.64(1.18)	13.30(1.54)[d]
41	33.55(4.46)	51.69(5.78)	6.14(1.40)	13.45(1.33)
44	41.19(5.05)	65.15(5.12)	7.48(1.88)	16.43(2.23)*
48	44.33(4.75)	70.27(4.81)	8.29(1.72)	18.29(2.77)
51	50.04(4.58)	77.27(5.07)	12.94(1.42)*	23.60(3.19)
58	55.04(5.56)	88.22(8.13)	16.56(2.09)	29.39(2.61)
61	60.37(4.98)	97.33(8.53)	23.56(1.96)	38.33(2.97)
65	64.50(5.82)	101.15(8.85)	24.76(1.34)	40.55(4.56)

* Significantly higher (P .05) from the previous value.
a,b Significant difference (P .05) between normal and abnormal cows (RIA).
c,d Significant difference (P .05) between normal and abnormal cows (EIA).
SE = Standard error.

Table (2)

Pregnancy diagnosis from milk progesterone levels by EIA Compared with pregnancy determination by rectal palpation

Days post-insemination	EIA positive			EIA Negative		
	Total NO of samples	Confirmed by palpation		Total No. of samples	Confirmed by palpation	
Single samples		No.	(%)		No.	(%)
18	21	17	80.9	15	11	73.3
19	14	13	92.8	13	10	76.9
20	19	18	94.7	25	19	76.0
21	13	12	92.3	15	12	80.0
22	11	10	90.9	11	10	90.9
23	11	10	90.9	6	5	83.3
24	10	9	90.0	10	8	80.0
25	19	19	100.0	9	6	66.7
Two Samples						
18,19	14	13	92.8	9	7	77.7
19,20	13	11	84.6	12	10	83.3
20,21	9	8	88.9	14	11	78.6
21,22	9	8	88.9	10	8	80.0
22,23	9	8	88.9	4	4	100.0
23,24	8	7	87.5	4	4	100.0
24,25	8	8	100.0	6	5	83.3
Three Samples						
18-20	12	11	91.7	7	5	71.4
19-21	6	5	83.3	6	4	66.7
20-22	9	8	88.9	10	9	90.0
21-23	6	5	83.3	4	4	100.0
22-24	7	6	85.7	4	4	100.0
23-25	6	6	100.0	4	4	100.0
Overall	35	32	91.4	29	23	79.3

PROGESTERONE DAIRY COWS

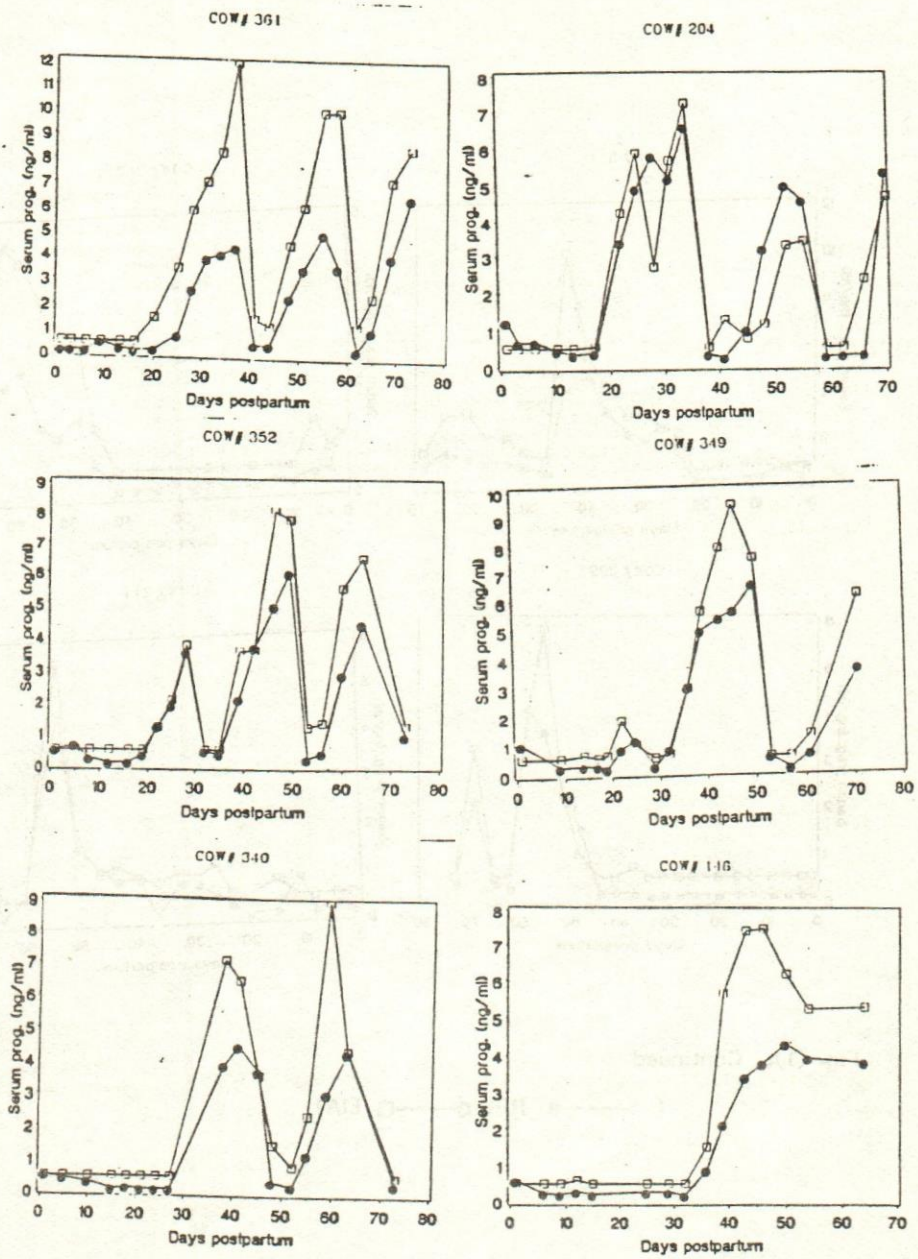

Fig. (1): Postpartum progesterone profile of individual cows

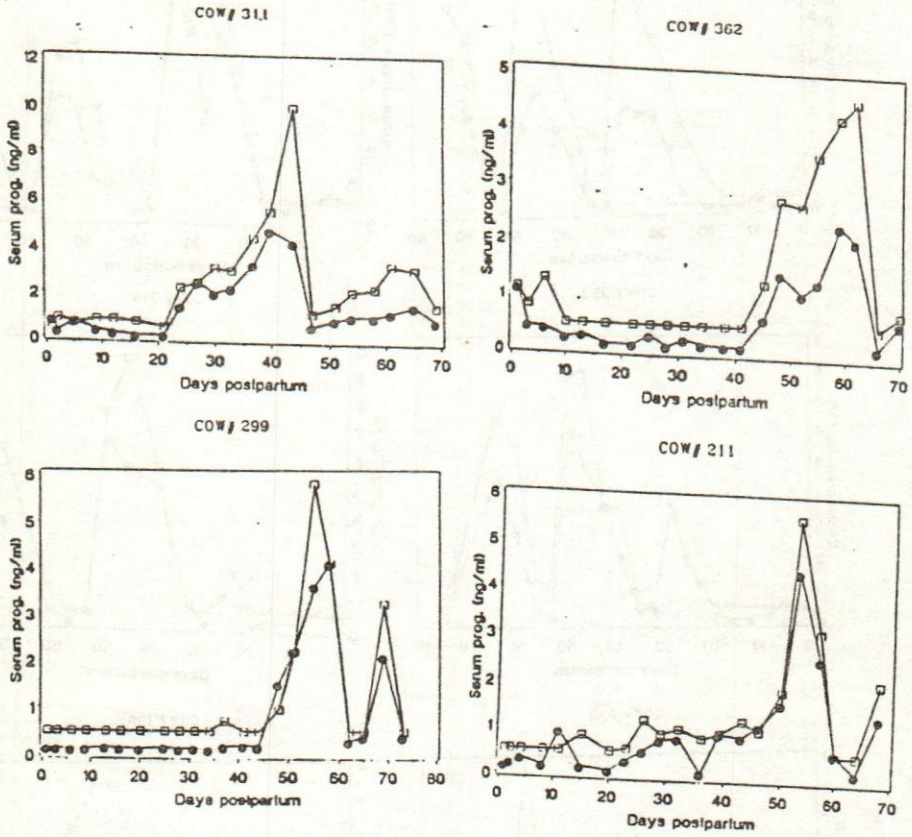

Fig. (1): Continued

(RIA EIA)

PROGESTERONE DAIRY COWS

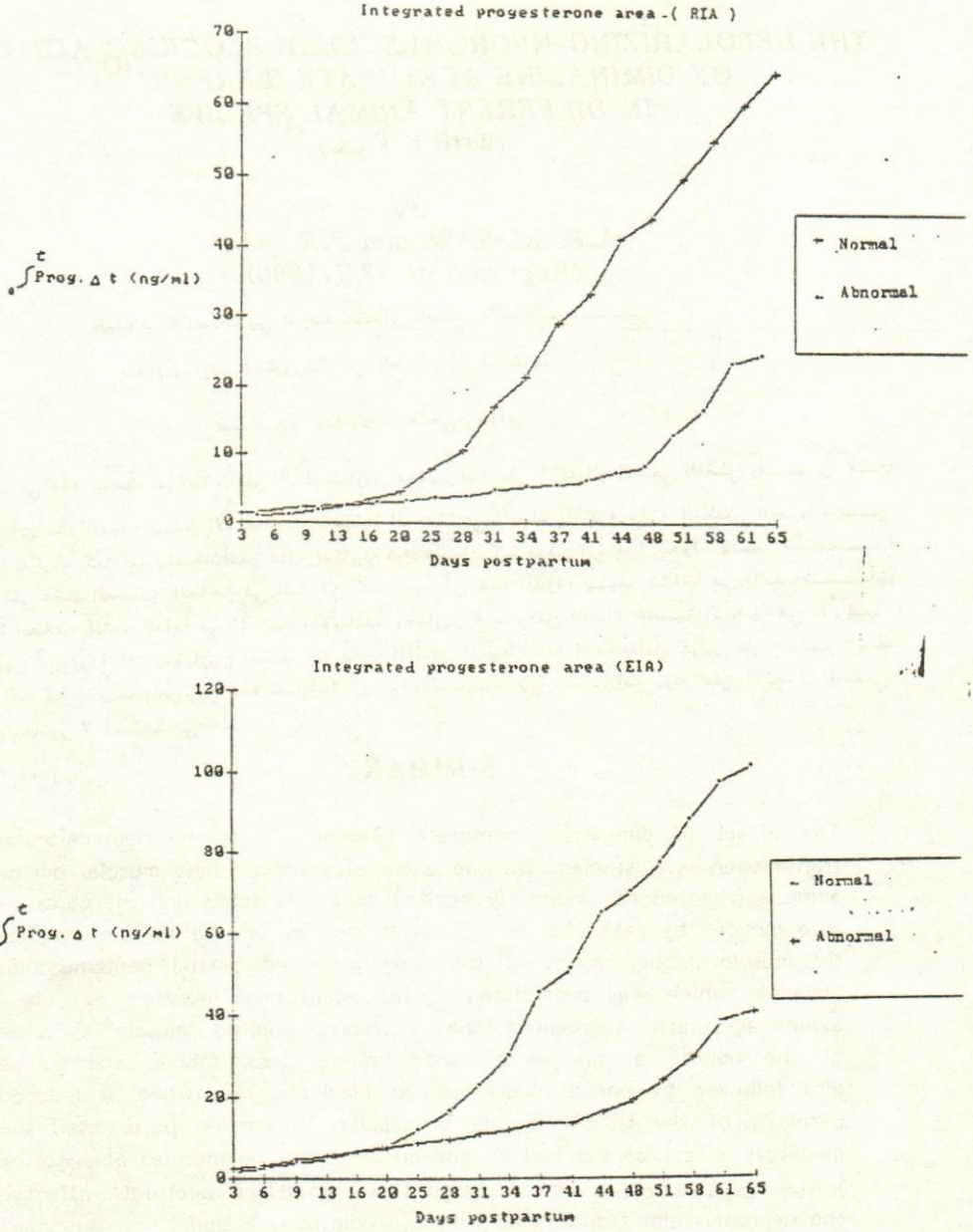

Fig. (2): Integrated progesterone area of 7 normal and 3 abnormal cows