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USE OF MILK PROGESTERONE RADIOIMMUNO ASSAY AND ENZYME-IMMUNOASSAY IN TEH DIAGNOSIS OF PREGNANCY IN BUFFALOES

BY

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SUMMARY

In veiw of comparing between milk progesterone radioimmunoassay and enzymeimmunoassay for diagnosis of pregnancy in buffaloes, 140 whole milk samples were collected from 20 cows on days 18th, 19th, 20th, 21st 22nd, 23rd and 24th after mating. The milk samples were analysed for progesterone with RIA and EIA, Applicability, reliability and the values of progesterone measured by both methods were compared. This study indicated that day 20th, 21th or 22nd is the most reliable for performing this analysis for pregnancy diagnosis in buffaloes.

INTRODUCTION

Pregnancy diagnosis at early stage as possible is of crucial importance in the establishment and maintenance of optimal reproductive performance of the farm animals. The principal characteristics of an ideal method for pregnancy diagnosis are that it should be accurate, inexpensive, easily and quickly performed. Although rectal examination is the most reliable method for pregnancy diagnosis in cattle and buffalo, it is not possible until at least six weeks of gestation. Heap et al., (1973) suggested that progesterone concentration in milk might provide means of early pregnancy diagnosis in lactating cows. Most of the early progesterone measurements in milk were carried by radioimmunoassay (Batra et. al., 1979 and Heinonen, 1988) which restricted its practical use to specialized laboratories with the capacity to use radioisotopes.

The objective of the curent study was to compare between radioimmunoassay (RIA) and enzymeimmunoassay (EIA) for quantifying the milk progesterone as a diagnostic aid for identifying the non pregnant buffalo cows.

MATERIALS AND METHODS

1. Animal and Management

Multiparous buffalo cows (n=35) belonging to an experimental herd in the Faculty of Agriculture, Cairo University were selected for this study. The buffalo cows were between 90-160 days (mean 110±9 days) after normal parturition. All these buffaloes were housed in semi-open yards and received a formulated ration and green fodder according to body weight and milk yield. The animals were milked twice daily at 6 a. m. and 3 p. m.

Ovrian cyclicity was monitored by rectal palpation of the ovaries and quantifying the progesterone in whole milk samples as previously described by Ghoneim et. al., (1992). Relaying on rectal examination and evaluation of milk progesterone, 20 animals were diagnosed as cyclic and the rest were acyclic. A schedule of double I/M injection of 500µg Cloprostenol (Estrumate ICI) at 10 days apart was carried out on cyclic animals. Twenty four hours after the second injection of Estrumate, animals were checked for oestrus twice daily for 120 hours using intact buffalo bull. In each observation the bull was permitted to run with the females for 30 minutes at least and allowed to serve the oestrous buffalo cows.

2. Milk Progesterone:

From day 18th after mating (day 0=day of mating) up to the day 24th, daily milk sample was collected from each animal. Every sample was 10 ml of whole milk collected in screw capped bottle and preserved with 100 mg sodium azide tablet (merck Art 6687 Darmstadi, Garmsiadi, Germany). The samples stored at -20°C until progesterone assay was performed.

3. RIA for Determination of Milk Progesterone

Concentrations of progesterone were determined in duplicate 25 µl aliquots of whole milk using the double antibody technique as described earlier by Dobeli, (1980).

4. EIA for Determination of milk Progesterone

Concentrations of progesterone were determined by EIA as previously described by Ghoneim (1989). In Briefly, each well of microtiter plate was coated with 100µl working solution of antiserum against progesterone 7 α carboxyethlthioether BAS (institute voor Veeteel tkundig Onderzoek, Schoonoord, Driebergsweg, 10D, Postbus 501, 3700 AM Zeist, The Netherlands). 100µl of milk samples diluted 1:200 in assay buffer or 100 µl of progesterone standard solution were pipetted into appropriate wells of the coated microtiter plates. To each well, 25µl of progesterone -6B-ol-hemisucciate-hourse Radish Peroxidase (institute voor Veeteel okundig nderzoek, Schoonoord, Driebergseweg, 10D, Postubs 501, 3700 AM Zeist, The Netherlands) is added. The plate was incubated for 1 hour at 37°C in the dark. After washing 5 times with 300 µl of 0.05% Tween 80 in aqua bidest, 100µl of peroxidase substrate solution were added to each well. The enzyme reaction running for 40 min. in the dark at room temperature then was stopped by addition of 50µl of sulphuric acid 4N and optical density was read. Concentration were calculated by computer program using linear regression after logit/log transformation.

5. Statisitcal Analysis

Statistical analysis was applied according to Snedecor and Cohran (1982).

RESULTS

Reliability of RIA EIA:

A. Specificity:

Specificity of the antiserum in RIA as well as EIA system were examined by testing the cross reactivities with steroids of structurally related steroid.The results of cross reactivites were presented in Table 1 and 2.

Table (1): Cross reactivities of progesterone antiserum in RIA system.

Progesterone	1.00
Δ 5 pregnene 3 β-ol-20 one	0.005
11 β-hydroxyprogesterone	0.29
11 α -hydroxprogesterone	0.054
17-hydroxyprogesterone	0.013
Testosterone	0.004
Δ 4- Androstene - 3, 17- dione	<0.001
Estrone 17 β -Estradiol, Estriol	<0.001
Cholesterol	<0.001

Table (2): Cross reasctiveles of progesterone antiserum in EIA system.

Progesterone	1.00
11α- hydroxyprogesterone	0.056
5 α - pregnane 3, 20 dione	0.25
Δ 5-prgnane-3 β ol-20 one	0.017
17α - hydroxyporgesterone	<0.0006
Cortisol	0.001
Estrone, Estradiol -17 β	<0.0006
Testosterone	<0.0006
Corticosterone	<0.0006
11 desoxy-corticostierone	<0.0006

B. Sensitivity:

The sensitivity of EIA (5 pg/well) almost equal to the one of RIA (10 pg/ube).

C. Precision:

Two progesterone controls from pooled whole milk samples were selected for this purpose. The low control contained about 1.5 ng/ml and the high control contained about 3.5 ng/ml. The control were assayed in duplicate at four differe

...ts of each RIA and EIA and the mean intra ...ay coefficient for each were determined. A ...n duplicate determination form each quality ...rol in nine different RIA as well as EIA were ...domly selected to determine the mean ...er-assay coefficients of variation in nine ...ferent tests. The results of precision for RIA ...d EIA are presented in Table 3.

Comparison of progesterone values estimated by RIA versus EIA:

A total of 140 milk sample were taken for comparison of progesterone estimation by RIA and EIA. The values obtained by the two methods were well correlated (r=0.95, P<0.05). The linear equation between these two methods (Fig. 1)

Table (3): Intra-and inter assay precision for RIA and EIA.

Quality control	Mean	RIA intra-assay CV (%)	inter-assay CV (%)	Mean CV (%)	EIA Intra-assay CV(%)	Inter-assay
Low	1.56	6.3	12.9	1.44	11.1	12.8
High	3.74	6.8	12.1	3.42	9.8	12.7
Overall mean CV		6.55	12.5		10.45	12.5

The results of precision indicated that in RIA system, the between assay CV (12.5%) was of the same order of the magnitude as in the EIA system (12.5%) as mentioned by Maneewan Kamonpatana (1979). However, the within assay CV of EIA is rather high (10.45) as compared to the corresponding RIA value (6.5%). This phenomena was recorded by Joyce et. al., (1977).

shows that RIA method measured slightly more progesterone than EIA values (Y=0.893 x + 0.564 x = RIAvalue Y= EIA value). This observation was also recorded by Ghoneim (1989). On the other hand, Prakash et. al., (1990) recorded higher values of progesterone when determined with EIA rather than RIA.

Table (4): Results of milk progesterone RIA and EIA for pregnancy diagnosis at different days after mating.

Days after mating	Milk Progesterone RIA		MilkProgesterone EIA		Results of rectal Exam.
	No. of pregnant buffaloes	No. of non pregnant bufaloes	No. of pregnant buffaloes	No. of non pregnant buffaloes	
18	13	7	12	8	14 animals were non pregnant and 6 animals were pregnant
19	10	10	9	11	
20	8	12	8	12	
21	8	12	8	12	
22	8	12	8	12	
23	10	10	8	12	
24	11	9	9	11	

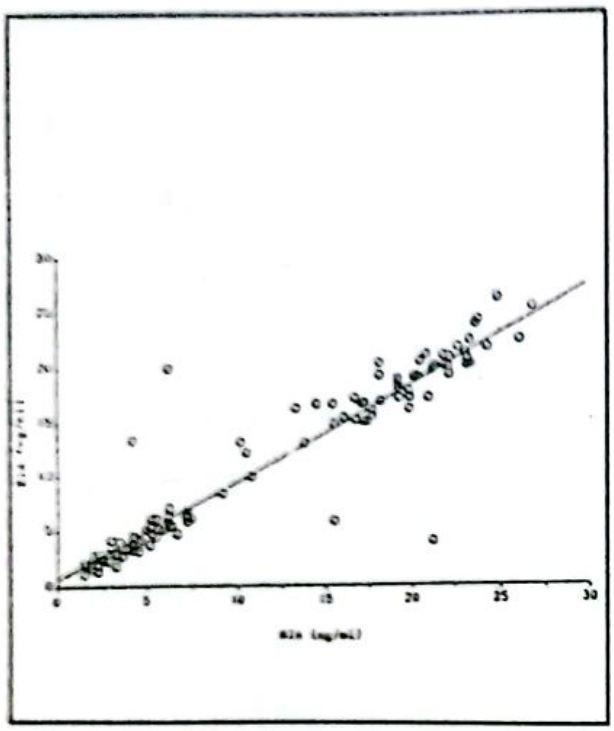

Fig. (1): Relationship between the concentrations of progesterone as measured by RIA and EIA system.

Pregnancy diagnosis in buffalo cows based on the estimation of milk progesterone by the use of RIA and EIA is presented in table 4.

DISCUSSION

Applicability of RIA and EIA:

Determining progesterone concentrations with RIA requires about 24 hours for analysis in addition to the time needed for shipping the samples. On the other hand, determining progesterone concentrations with EIA requires about 3 hours. Hence, buffalo cows that do not concieved after breeding and does not show oestrous signs due to silent heat which is frequent in buffaloes (Shah et.al., 1991) must wait another 21 days before being rebred if they ar examined for progesterone level by RIA. These missed breeding opportunities result in longer intervals from parturition to breeding. Since its endpoint determination consists of measuring the optical density, the equipment for EIA may be somewhat less expensive than RIA. This merit makes the EIA more applicable in developing countries. In the EIA system most of the working steps are easily automated but can also be run manually using adequate pipetting devices (See Ghoneim, 1989). The use of radioacativity prohibits any application of RIA as a field test and gives the chance for pollution of the environment.

Using of RIA and EIA for determining the level of milk progesterone as a method for pregnancy diagnosis:

Return or non return to oestrus 21 days after services is of course the first step for identifying the non pregnant animals. The silent heat which is more frequent in buffalous (Shah et. al., 1991) is as obsticle which hinder the relaying on this phenomena for identifying the non pregnant animals. Measurement of progesterone offers a better alternative for identifying the non pregnant buffaloes. On the basis of the concentrations of the progesterone, animals were classified as pregnant (≥ 7 ng/ml) or non pregnant (< 7 ng/ml). Table 4 shows the number of pregnant and non pregnant animals based on determination of progesterone with RIA and EIA on days 18th, 19th, 20th, 21st. 22nd, 23rd and 24th. Comparing the results of RIA milk progesterone analysis with that of rectal examination revealed overestimation of pregnancy rate of 123.3 %, 66.6%, 33.3%, 33.3%, 33.3%, 66.6% and 83.3% on days 18th, 19th, 20th, 21st, 22nd, 23rd and 24th respectivley.

Underestimation of the non pregnancy rate of 50%, 28.5%, 14.2%, 14.2%, 28.5% and 35.7% was recorded on days 18th, 19th, 20th, 21st, 22nd, 23rd and 24th respectively. On the other hand, comparing the results of EIA milk progesterone analysis with that of rectal examination indicatted overestimation of pregnancy rate of 100.0%, 50.0%, 33.3%, 33.3%, 33.3%, 33.3% and 50% was recorded on days 18th, 19th, 20th, 21st, 22nd, 23rd and 24th respectively. Underestimation of the non pregnancy rate of 42.8%, 21.4%, 14.2%, 14.2% and 21.4% on day 18th, 19th, 20th, 21st, 22nd, 23rd and 24th respectivley, when EIA milk progesterone analysis was used. Aformentioned results indicated that in both RIA and EIA milk progesterone analysis for pregnancy diagnosis, the day 20th, 21st or 22nd was the most reliable for performaing this analysis for pregnancy diagnosis in buffaloes.

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