

PLASMA AND MILK PROGESTERONE CONCENTRATIONS AND EARLY PREGNANCY IN ZEBU COWS

M. G. S. Alam¹ and A. Ghosh²

Department of Surgery and Obstetrics, Faculty of Veterinary Science
Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

Summary

In an effort to confirm true oestrus and to detect early pregnancy in Zebu cows (*Bos indicus*), sequential blood and milk samples were collected at the day of insemination (Day 0) and days 14, 20 and 24 after insemination. Progesterone was determined in skimmed milk and plasma by solid phase radioimmunoassay (RIA). Of the cows thought to be in oestrus plasma, (n = 46) and milk (n = 58) samples demonstrated low progesterone concentrations (0.99 ± 0.11 and 2.02 ± 0.14 nmol/l) in 42 (91%) and 52 (90%) cases respectively. Thirty two (76%) and 30 (71%) cows were thought to be pregnant by plasma progesterone RIA (20.23 ± 1.03 and 20.48 ± 1.01 nmol/l) at days 20 and 24 respectively. At the same period, 40 (77%) and 37 (71%) cows were thought to be pregnant by milk progesterone RIA (27.82 ± 1.28 and 28.02 ± 1.27 nmol/l). Assuming 100% accuracy for rectal examination of pregnancy diagnosis between days 60-65 postservice, the RIA was found to be 84% and 90% accurate for plasma and 84% and 92% accurate for milk at day 20 and 24, respectively. All cows thought to be non pregnant by progesterone measurement were correctly diagnosed. Progesterone assay at 24 days after oestrus may therefore be accurate for early diagnosis of pregnancy in Zebu cows.

(Key Words: Progesterone Assay, Pregnancy, Milk, Plasma, Zebu Cows)

Introduction

The introduction of modern methods of oestrus detection (Orihuela et al., 1983) and early pregnancy diagnosis (Worsfold et al., 1987) are essential for the profitable cattle industry. The concentrations of progesterone in peripheral circulation directly reflect the functional activity of corpus luteum (Eger et al., 1989). Measurement of plasma and milk progesterone concentrations may offer a convenient method for selecting animals for artificial insemination (AI) where clinical signs of oestrus are not obvious (Reimers et al., 1985; Eddy and Clark, 1987). The determination of pregnancy between days 20-24 post breeding has been achieved by measuring plasma and milk progesterone concentrations for over 10 years (Hooth et al., 1979; Laing et al., 1979; Adeyemo, 1989; Nebel et al., 1989), however there

is no information on progesterone profiles at the time of AI and during early pregnancy of Zebu cows in Bangladesh. A study was therefore undertaken to determine ovarian activity at the time of AI and during early pregnancy in Zebu cows using solid phase progesterone RIA kits.

Materials and Methods

Sixty-four multiparous mixed breeding Zebu cows aged between 4-8 years and weighing between 150-225 kg were studied. The animals were housed in the stalls under natural lighting with traditional feeding and were allowed to graze for about 5 hours each day. Cows were observed throughout the study at 07:00, 17:00 and 22:00 hours for 30 minutes on each occasion. Oestrus was assessed by the mounting behaviour of the teaser bull. Cows were inseminated within 12-18 hours after the onset of standing oestrus. To standardize the data, the day of oestrus/insemination was designated as day 0. Blood and milk samples (10 ml) were taken between 14:00-16:00 hours from 40 animals at the day of oestrus and on days 14, 20, 24 post service. Milk samples from a further 18 non-pregnant animals and

¹Address reprint requests to Dr. M. G. S. Alam, Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh.

²Veterinary Assistant Surgeon, Tojumuddin, Bhola, Bangladesh.

Received September 1, 1993

Accepted November 3, 1993

blood samples from 6 dry cows were collected as before.

Blood samples were collected with a syringe containing 0.25 ml of heparinized saline (500 i.u. sodium heparin/100 ml, 0.9% saline) by jugular venipuncture. Immediately after collection it was centrifuged at 1,000 g for 10 minutes and the plasma was harvested and stored at -20°C until progesterone assay.

Foremilk samples were placed in a collecting tube containing a sodium azide tablet as preservative. These were stored at -20°C until assay. One or 2 days preceding assay the sample was thawed and fat particles were removed by centrifugation at room temperature at 1,000 g for 10 minutes. The supernatant fat particles were discarded and the defatted milk samples were collected and stored at -20°C until assay.

Plasma and defatted milk samples were analyzed for progesterone concentrations by solid phase RIA as described by the Agriculture laboratory, joint FAO/IAEA Division, Seibersdorf, Austria. Briefly, plasma and/or milk (0.1 ml in duplicate) samples were placed into antibody coated tubes, incubated at room temperature for 4 hours with 0.1 ml of ^{125}I progesterone. After decanting, all tubes were counted individually in a portable Gamma counter (Type 6-90; Mini Instruments; Burnham on Crouch, England) for one minute. The within and between assay coefficient of variation were of 9% ($n = 16$) and 9% ($n = 5$) for plasma and 6% ($n = 13$) and 9% ($n = 5$) for milk, respectively. The percent binding at 0 dose level was 28 ± 2.3 ($n = 8$).

Ovarian activity was assessed on the basis of progesterone concentrations at the time of service and early pregnancy period.

Clinical examinations of the reproductive organs were performed by palpating per rectum on the day insemination and between days 60-65 post insemination. The accuracy of positive diagnosis was calculated as the percentage of cows diagnosed pregnant by progesterone concentration at day 20 and 24, that were reconfirmed by rectal examinations of the reproductive organs between days 60-65 post insemination. The cows were classified as pregnant or non-pregnant.

For plasma and milk, minimum progesterone concentrations considered positive for the diagnosis of pregnancy were of 5 nmol/l and 8 nmol/l, respectively.

The accuracy of positive pregnancy diagnosis was calculated as the percentage of cows with elevated progesterone concentrations at day 20 and 24, that were subsequently confirmed pregnant by rectal examination. The accuracy of a negative diagnosis was calculated as the percentage of cows with low progesterone concentrations which were subsequently confirmed to be non pregnant by rectal examination. The accuracy of positive diagnosis of pregnancy on the basis of milk and plasma progesterone concentrations at day 20, 24 was analysed statistically by proportions test (Snedecor and Cochran, 1967). Average progesterone concentrations for a specific day were expressed as mean \pm standard error of mean.

Results

The progesterone profiles from the samples obtained on day 0, 14, 20 and 24 after insemination are presented in figure 1. Cows were divided into 4 groups on the basis of the pattern of progesterone concentrations.

Group 1, pregnant

Progesterone concentrations which were basal on day 0 were elevated on days 14, 20 and 24. At day 20, plasma and milk progesterone concentrations were 20.23 ± 1.03 nmol/l and 27.82 ± 1.28 nmol/l, respectively. Values at day 24 were 20.48 ± 1.01 nmol/l and 28.02 ± 1.27 nmol/l, respectively. There was no significant ($p > 0.05$) difference between the concentrations of progesterone in plasma or milk observed at day 20 or day 24.

Group 2, non-pregnant diagnosis at day 20

Mean concentrations of plasma and milk progesterone which were initially basal increased to 18.2 ± 1.14 nmol/l and 26.12 ± 1.76 respectively at day 14 post-insemination. There was no significant ($p > 0.05$) difference in progesterone concentrations at day 14 between pregnant (Group 1) and non pregnant (Group 2) cows. However in non-pregnant cows the progesterone concentrations subsequently declined to the initial values obtained on the day of insemination (plasma and milk progesterone concentrations 1.72 ± 0.31 nmol/l and 3.13 ± 0.36 nmol/l, respectively). The differences in progesterone concentrations between pregnant (Group 1) and non-pregnant cows

PROGESTERONE EARLY PREGNANCY IN ZEBU COWS

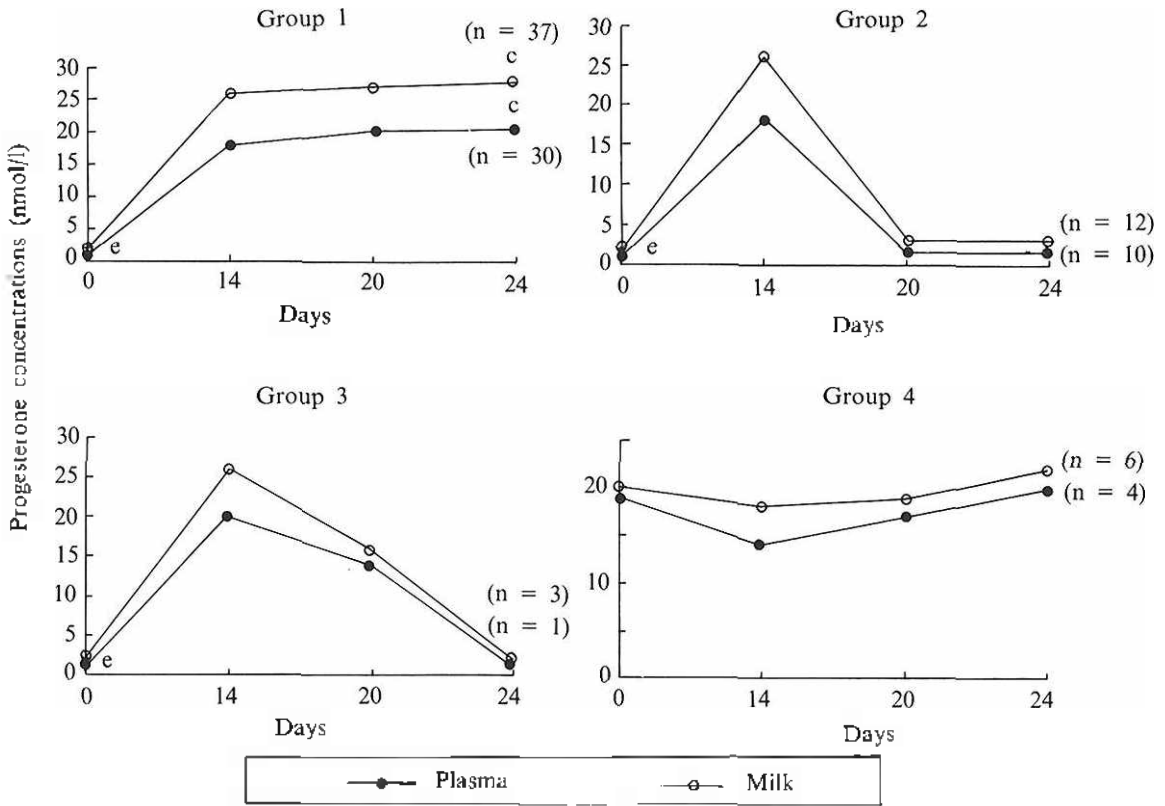


Figure 1. Postbreeding plasma and milk progesterone concentrations (nmol/l; mean \pm sem) in Zebu cows. (e = oestrus, c = conception, n = no. of cows)

(Group 2) were significant ($p < 0.01$) at days 20 and 24.

Group 3, non pregnant diagnosed at day 24

Plasma and milk progesterone concentrations increased up to day 14 (mean plasma and milk progesterone concentrations 20.0 ± 2.0 nmol/l and 26.17 ± 1.92 nmol/l respectively). Concentrations then started to decline but remained higher than the discriminating level between pregnant and non-pregnant cows at day 20, although they had declined to basal level at day 24. Progesterone concentrations were significantly different ($p < 0.01$) between pregnant (Group 1) and non-pregnant (Group 2) cows at day 24.

Group 4, oestrous behaviour during the luteal phase

Six cows exhibited behavioral oestrus and had elevated progesterone concentrations (mean 5 nmol/l for plasma and 8 nmol/l for milk), through-

out the sampling period. These cows were diagnosed non-pregnant on the day of AI and did not become pregnant to the insemination.

Of the cows thought to be in oestrus, plasma ($n = 46$) and milk ($n = 58$) samples obtained on the day of insemination demonstrated low progesterone concentrations in 42 (92%) and 52 (90%) of cows, confirming these cows to be in oestrus (mean plasma and milk progesterone concentrations were 2.0 nmol/l and 3.0 nmol/l respectively). Of these, only 32 (76%) and 30 (71%) animals were thought to be pregnant as assessed by plasma progesterone RIA at day 20 and 24 respectively (table 1). During the same period, 40 (77%) and 37 (71%) cows were thought to be pregnant by milk progesterone RIA (table 2). For determining accuracy of the laboratory diagnosis, manual palpation of the reproductive organs between days 60-85 post insemination were assumed to be 100% accurate. Based on this assessment the measurement of progesterone was

found to be 84% and 90% accurate for plasma samples and 84% and 92% accurate for milk samples obtained at day 20 and 24 respectively. Negative diagnosis was 100% accurate. The dif-

ferences between the accuracy of positive diagnosis for plasma and milk progesterone were insignificant ($p > 0.05$). The percentage of accuracy at day 20 and 24 were also insignificant ($p > 0.05$).

TABLE 1. DIAGNOSIS OF PREGNANCY BY PLASMA PROGESTERONE ASSAY IN ZEBU COWS ($n^1 = 46$)

	Days post-insemination			
	0	20	24	60-65
Observations (no)	42	41	46	46
True oestrus (no)	42			
True oestrus (%)	91.3			
Diagnosis +ve ² (no)		32	30	
Confirm +ve (no)				27 ^b
Error of +ve diagnosis (no)		5	3	
Error of +ve diagnosis (%)		15.6	10.0	
Accuracy of +ve diagnosis (%)		84.4	90.0	
Diagnosis -ve ³ (no)		4 ^{ac}	13 ^c	
Confirm -ve (no)				19 ^c
Missed non-pregnancy (%)		26.3 ^c	15.8 ^c	

Plasma progesterone was assayed by RIA on day 0, 14, 20 and 24 after insemination. For +ve and -ve diagnosis only the samples of day 0, 20 and 24 were considered. High progesterone concentrations (> 5 nmol) on day 0 was considered as non fertilized^a. Genital organs were palpated per rectum between days 60-65 for confirming positive diagnosis^b. In most cases (19^c) confirmed non pregnancy^c was estimated before days 60-65.

¹ Number inseminated. ² Positive. ³ Negative.

TABLE 2. DIAGNOSIS OF PREGNANCY BY MILK PROGESTERONE ASSAY IN ZEBU COWS ($n^1 = 58$)

	Days post-insemination			
	0	20	24	60-65
Observations (no)	52	52	58	58
True oestrus (no)	52			
True oestrus (%)	89.7			
Diagnosis +ve ² (no)		40	37	
Confirm +ve (no)				34 ^b
Error of +ve diagnosis (no)		6	3	
Error of +ve diagnosis (%)		15.0	8.8	
Accuracy of +ve diagnosis (%)		85.4	91.9	
Diagnosis -ve ³ (no)		6 ^{ac}	18 ^c	
Confirm -ve (no)				24 ^c
Missed non-pregnancy (%)		25.0 ^c	12.5 ^c	

Milk progesterone was assayed by RIA on day 0, 14, 20 and 24 after insemination. For +ve and -ve diagnosis only the samples of day 0, 20 and 24 were considered. High progesterone concentrations (> 8 nmol/l) on day 0 was considered as non fertilized^a. Genital organs were palpated per rectum between days 60-65 for confirming positive diagnosis^b. In most cases (24^c) confirmed non pregnancy was estimated before days 60-65.

¹ Number inseminated. ² Positive. ³ Negative.

Discussion

It is evident from this study that 91.30% and

89.66% cows were confirmed to be in true oestrus by plasma ($n = 46$) and milk ($n = 58$) progesterone RIA, respectively. Similarly 76.19%

71.43%, 76.19% and 71.43% cows were diagnosed pregnant by plasma and milk progesterone assay at day 20 and 24 respectively.

Determination of plasma and milk progesterone concentrations for the diagnosis of true oestrus and early pregnancy have been reported by several workers on cows of *Bos taurus* (Worsford et al., 1987; Eddy and Clark, 1987; Nebel et al., 1989) and *Bos indicus* species (Adeyemo, 1989). Such determination could play a substantial role in maintaining a desirable calving pattern by reducing the false pregnancy diagnosis.

High progesterone concentrations at the time of insemination of cows in the luteal phase is a common cause of low fertility. This may be a reflection of mid cycle follicular development (Alam and Dobson, 1986) or the animals were thought to be in oestrus. Moreover, expression of oestrous behaviour in 6% cases has been reported in pregnant cows (Thomas and Dobson, 1989). The wide variation in duration of oestrus may be due to breed peculiarities which may increase the chance of false oestrus detection (Gallina et al., 1982). The low mounting activity of Zebu cows may enhance the likelihood of false oestrus detection (Orihuela et al., 1983). Diagnosis of true oestrus at the time of insemination by plasma and milk progesterone assay may increase the accuracy of early pregnancy diagnosis because cows inseminated during the luteal phase may be incorrectly diagnosed as pregnant following analysis of samples at day 20 or 24 post insemination. Alternatively, the insemination of pregnant cows may result in the loss of embryo and consequently extends the calving interval.

Plasma progesterone concentrations of pregnant cows in this study was slightly higher than those of other report (Choi et al., 1989). The milk progesterone concentrations were found to be lower in European cows (Booth et al., 1979; Thomas and Dobson, 1989) where milk fat and/or feed ingredients may influence the concentrations of milk progesterone, but the results of this study are in agreement with Rainio (1987) who reported similar values in Hereford cows. However, elevated progesterone concentrations are indicative of the presence of luteal tissue, which does not always indicate the presence of a live foetus in the uterus. The wide variation in the concentrations of plasma and milk progesterone may be due

to animals of different breeds, laboratory procedures and environment.

The accuracy of pregnancy diagnosis in Zebu cows using milk and plasma progesterone determination demonstrated a potential use similar to reports in other bovine species (Laing et al., 1979; Adeyemo, 1989; Choi et al., 1989). The differences of accuracy of prediction at day 20 and 24 were not statistically different ($p > 0.05$). However, samples tested from day 24 post-service may be better than those at day 20, because cows with prolonged luteal activity may falsely be diagnosed pregnant on day 20 (Noakes, 1985).

The accuracy of prediction was less than 100% and may have been the result of early embryonic loss since early embryonic mortality may sometimes be associated with a decline of progesterone concentrations 30 days post-breeding (Noakes, 1985).

The 100% accuracy for the prediction of non-pregnant cows is similar to the report by Thomas and Dobson (1989), although it may have been expected that there would be a percentage of false negative case. This may have been negated in the present study by the values selected for discrimination between pregnancy and non-pregnancy (< 5 nmol/l progesterone in plasma and < 8 nmol/l progesterone in milk).

Solid-phase progesterone RIA thus would appear to be useful for the diagnosis of pregnancy in Zebu cows.

Acknowledgements

The authors thank the International Atomic Energy Agency, Vienna, Austria (Contract no. 5214/RB) and Bangladesh Agricultural Research Council, Dhaka for financial and technical support. Gratitude is extended to Mr. Parash Chandra Mudok, Department of Agricultural Statistics, Bangladesh Agricultural University, Mymensingh for statistical analysis.

Literature Cited

- Adeyemo, O. 1989 Application of plasma and milk progesterone assay in pregnancy diagnosis in White Fulani (Zebu) cattle. *Anim. Reprod. Sci.* 19: 205-208.
- Alam, M. G. S. and H. Dobson. 1986. Effect of various veterinary procedures on plasma concentrations

- of cortisol, luteinizing hormone and prostaglandin F_2 alpha metabolite in the cow. *Vet. Rec.* 118: 7-10.
- Booth, J. M., Davis, J. and R. J. Holdsworth 1979. Use of the milk progesterone test for pregnancy determination. *British Vet. J.* 135:478-488.
- Choi, K. G., B. K. Kang and C. G. Lee 1989. Application of progesterone measurement for fertility control in Korean native cattle. FAO/IAEA Research Co-ordinating Meeting on Strengthening Animal Reproduction Research in Asia, Malaysia, 22-23 May.
- Eddy, R. G. and O. J. Clark. 1987. Oestrus prediction in dairy cows using ELISA progesterone kit. *Vet. Rec.* 120:31-34.
- Eger, S., M. Shemesh, H. Schindler, S. Amir and R. H. Foot 1989. Characterization of short luteal cycles in early post partum period and their relation to reproductive performance of dairy cows. *Anim. Reprod. Sci.* 16:215-224.
- Gallina, C. S., A. Calderon and M. McCloskey. 1982. Detection of signs of oestrus in the Charolais cows and Brahma cows under continuous observation. *Theriogenology.* 17:485-498.
- Leung, J. A., S. A. K. Eastman and J. C. Bouflower. 1979. The use of progesterone concentrations in milk and plasma for pregnancy diagnosis in cattle. *British Vet. J.* 135:264-269.
- Noakes, D. E. 1985. Pregnancy diagnosis in cattle. In *Practice* 7:46-51.
- Nebel, R. L., D. L. Altemose, T. W. Munkittrick, D. J. Sprecher and M. L. McGilliard. 1989. Comparisons of eight commercial on farm milk progesterone tests. *Theriogenology.* 31:753-764.
- Oltner, R. and L. E. Edqvist. 1981. Progesterone in defatted milk: its relation to insemination and pregnancy in normal cows as compared with the cows on problem farm and individual problem animal. *British Vet. J.* 137:78-87.
- Ortuola, A., C. Gallina, J. Eschbar and E. Riquelme. 1983. Estrus behaviour following prostaglandin F_2 alpha injection in Zebu cattle under continuous observation. *Theriogenology.* 19:795-809.
- Rainio, V. 1987. Whole milk progesterone analysis used for pregnancy diagnosis in beef cattle. *Acta Vet. Scan.* 28:249-250.
- Reimers, T. J., R. D. Smith and S. K. Newman. 1985. Management factors affecting reproductive performance of dairy cows in the northern United States. *J. Dairy Sci.* 68:963-972.
- Snedecor, G. W. and W. G. Cochran. 1967. *Statistical methods*, 6th Edn. The IOWA State University Press.
- Thomas, I. and H. Dobson. 1989. Oestrus during pregnancy in the cows. *Vet. Rec.* 124:387-390.
- Wersfold, A. I., J. M. Rooth, P. M. Wells, A. C. Huddart and C. J. Stanleys. 1987. The evaluation of a new rapid milk progesterone test as an aid to improving dairy herd fertility. *British Vet. J.* 143:83-87.