PROGESTERONE CONCENTRATIONS MEASURED BY RADIOIMMUNOASSAY IN BLOOD PLASMA AND IN FAT-FREE MILK OF GIR BREED COWS (BOS INDICUS). DETERMINATIONS DURING OESTRUS CYCLE. EARLY PREGNANCY DIAGNOSIS.

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MADUREIRA, E.H.; BARNABE, R.C.; PINTO, P.A.; BARNABE, V.H.; MARTINS SOBRINHO, E.O. Progesterone concentrations measured by radioimmunoassay in blood plasma and in fat-free milk of Gir breed cows (*Bos indicus*). Determinations during oestrus cycle. Early pregnancy diagnosis. *Braz. J. vet. Res. anim. Sci.*, São Paulo, *27*(2):247-253, 1990.

SUMMARY: Progesterone concentrations were measured by radioimmunoassay in 137 fat-free milk and in 138 blood plasma samples obtained three times weekly during 17 estrous cycles from 12 zebu Gir cows, raised in range conditions at the "Centro Intraunidade de Zootecnia e Indústrias Pecuárias Fernando Costa", Pirassununga, State of São Paulo, Brazil. Profiles of progesterone concentrations for early pregnancy diagnosis were also examined on samples taken 23 days after natural service. Correlations were found between frozen and cooled milk samples (r = 0.92), as well as between milk and blood plasma progesterone levels (r = 0.63). Variations during estrous cycle were detected, with lower values in folicular phase (less than 1.49 nmol/l in plasma and 2.61 nmol/l in milk) and peak values in luteal phase (from 15.60 to 21.30 nmol/l in plasma and 5.91 to 11.37 nmol/l in milk). All pregnancy and non pregnancy diagnosis were confirmed by rectal palpation, when 9.54 nmol/l in plasma and 3.18 nmol/l in milk discriminations values were utilized. From the combined data of 24 oestrus observations and progesterone profiles (29 oestrus) it was found that 17.2% of failure occured in oestrus detection.

UNITERMS: Progesterone; Milk; Blood plasma; Oestrus cycle; Early pregnancy diagnosis; Radioimmunoassay; Zebu cattle

INTRODUCTION AND LITERATURE

Progesterone concentrations in plasma and milk can be used to objectively determine ovarian function in female mammals. In cattle, single samples on the day of insemination can also be used to indicate if the timing is appropriate, and samples at 21 or 23 days after mating can accurately reflect non-pregnancy (CAVESTANY & FOOTE [5], 1985). Sequential samples at weekly or more frequent intervals can show the time of sexual maturity in growing females and the initiation of ovarian activity in recently parturient animals. The patterns of plasma and milk progesterone concentrations throughout the estrous cycle are similar in *Bos taurus* dairy cows. In a recent review, DOBSON & KAMONPATANA [6] (1986) pointed out to the need of having more information on milk progesterone profiles in zebu cattle. Also, no milk values have ever been reported for continuously suckled cows of any type of breed.

This study was conducted to determine plasma and milk progesterone concentrations during estrous cycle and, 23 days after mating, in Gir cows. In addition, the effect of milk storage at 4 or -18°C, the correlation between blood and milk values, and the practical applications of progesterone monitoring were investigated.

MATERIAL AND METHOD

Experimental animals

Twelve Gir suckled cows, in good body conditions, all at least 60 days after parturition, at the "Centro Intraunidade de Zootecnia e Indústrias Pecuárias Fernando Costa", Pirassununga, São Paulo State, Brazil, were kept on improved grass pasture and sampled regularly from April to July, 1987. A teaser bull was with

the cows throughout the study and the herdsmen recorded any observed hetero or homossexual behavior.

Sampling Procedure

Milk and blood samples were collected from each cow every Monday, Wednesday and Friday for a period from one estrous until the next observed period of sexual activity, producing a total of 137 samples. One additional sample was obtained at 23 days after a confirmed mating. Cows were separated from their calves at sampling time and placed in a restraining chute so the samples could be obtained. Milk was collected directly into a 10 ml tube containing one sodium azide tablet. Immediately after collection the samples were chilled and subsequently centrifuged (room temperature, 2300 g, 15 min.) to remove fat. The skim milk was normally stored at -18°C, but 28 individual collections were divided into two portions and stored at 4 or -18°C until assayed.

Blood was collected into Vacutainers with EDTA, chilled immediately in ice water and centrifuged to obtain plasma. The plasma was stored at -18°C until assayed.

Progesterone Assay

Progesterone concentrations in plasma and skim milk were determined using the FAO/IAEA kit (Seibersdorf, Austria).

The sensivity was .92nmol/l and coefficients of variability were 7.6 and 8.0% for intra and inter-assay, respectively.

Statistical Methods

A "Z" test was used to test for differences between chilled and frozen storage methods. The correlation between the two storage conditions and between milk and plasma values were calculated (BERQUÓ et alii [4], 1980).

RESULTS

Comparison of Milk Storage Temperatures

The progesterone concentrations in skim milk samples are given in Tab. 1. There was very close correlation between refrigerated and frozen samples ($r = .92$, $P < .05$) (Fig. 2).

Progesterone Profiles

During the follicular phase, progesterone concentration was very low in both milk and plasma (Fig. 1). The actual values for samples collected when cows were in oestrus ranged from undectetable (<.92 nmol/l) to 1.49 nmol/l in plasma and from undectetable to 2.61 nmol in milk. Maximum luteal phase values were found between days 10 to 18 in individual animals. The means were 18.45 ± 5.37 and 8.64 ± 5.16 nmol/l in plasma and milk respectively. The correlation between milk and plasma was .63 ($p < .05$).

Pregnancy Diagnosis Results

Progesterone values in samples taken on day 23 after mating were obviously clustered into a high and low group. For plasma, these were between 11.64 to 39.36 nmol/l and the low values were from undectetable to 3.13 nmol/l. Similar ranges in milk were 3.21 to 12.8 nmol/l for the elevated values and all undectetable in the others. Animals in the high group (n=9) were presumed pregnant, while the others (n=3) were considered to be non-pregnant. Subsequent rectal examination confirmed the diagnosis in all animals.

Estrus Detection Efficiency

The progesterone profiles indicated that the 12 cows had a total of 29 follicular phases during the sampling period. Estrus detection were examined by herdsman to see how many occurrences of estrus behavior were recorded. In 24 instances recording of sexual behaviour corersponded with the periods when progesterone was basal, for a detection efficiency of 88.0%. No heat signs were detected when progesterone was elevated.

DISCUSSION

Comparison of Milk Storage Temperatures

Progesterone profiles in chilled and frozen milk did not differ significantly ($P < .05$) so either method of storage is satisfactory. These results agree with BALL & POPE [3] (1976) who found no effect of storage condition on *Bos taurus* milk samples.

In a previous trial, a considerable number of samples were lost because of coagulation during storage. These were maintained at 45 °C for approximately 2 months, and this extended period may have contributed to the problem. However, these samples had been collected from dairy cows, some of them may have been affected with chronic mastitis. The

increased somatic cell counts in mastitic milk may have contributed to coagulation during storage. (*)

Ideally 10 ml samples should be collected directly in the vials containing a preservative tablet and placed in refrigerator immediately. Fat should be removed not latter than one week after collection and the fat-free sample maintained at 4 °C or frozen (-18 °C) until assayed. Frozen storage would be preferred since this provides a much greater safety margin. If storage is in refrigerators, assays should be performed as quickly as practical since chance of coagulation may increase with time. (*)

Plasma Progesterone Profiles

Mean progesterone concentrations are in accordance with most values reported elsewhere (STABENFELDT et alii [17], 1969; DOBSON et alii [7], 1975; DOBSON & KAMONPATANA [6], 1986). Although there are reports in the literature that indicate low progesterone values in zebu cattle (AGARWAL et alii [2], 1977; ADEYEMO & HEATH [1], 1980; RANDEL [14], 1984). The present study does not support this information, perhaps the level of nutrition in the experimental animals, or a defficient handling of the sample prior to assay contribute to these low progesterone values in the latter studies.

Milk progesterone values for zebu cows have previously not been available (DOBSON & KAMONPATANA [6], 1986). Our results indicate that at least for the Gir breed, skim milk and plasma progesterone concentrations are similar to *Bos taurus*. Possibly the depressed plasma values recorded in some *Bos indicus* studies resulted from failure to chill the samples quickly or nutritional defficiencies in the experimental cows. Several reports indicate that low plasma progesterone may be associated with low B-carotine (JACKSON et alii[11], 1981 e FRISH et alii [8], 1987).

Comparison of Plasma and Milk Values

The concentrations in milk and plasma were significantly correlated (r = .63). This values was lower than previously reported coefficients of .91 (POPE et alii [13], 1976) and .88 (DOBSON et alii [7], 1975) for groups or the range of .675 to .88 for individual cows (GIL et alii [9], 1980). However, these studies involved comparison of plasma and whole milk progesterone milk concentrations in *Bos taurus* dairy cows. Skim milk, as used in this study, has a substantially lower concentration than whole milk and this may have influenced the relationship. Also, this study used milk samples from suckled beef cows and the continued nursing could have influenced progesterone transfer from plasma to milk.

Plasma values were 2.4 times higher than skim milk which agree with POPE et alii [13] (1976).

Both profiles (Fig. 1) are typical of the cyclic patterns in cows with close agreement apparent for most points. As exception is the transition phase from mid-luteal until early follicular days. Plasma concentration are still quite high on day 17 with considerable variance. This resulted from inclusion of some cows with 22 to 25 day cycles that still had high progesterone concentrations and others which were already basal and entering their next estrus period. Irregular estrous cycles have been reported for post-partum Afrikaner cattle specially if they start cycling before 100 days after calving (WELLS et alii [19], 1981).

Pregnancy Diagnosis

Various discriminatory plasma values have been suggested as criteria for pregnancy and non-pregnancy decisions, but most range was between 1.5 (POPE & HODGSON JONES [12], 1975) and 2.5 (SHEMESH et alii [16], 1973) and 3.0 ng/ml (WISHART et alii [20], 1975). In this study cows with progesterone below 3.18 nmol/l (1 ng/ml) on day 23 were considered non pregnant and subsequent rectal examination indicated that the diagnosis was correct in all cases. The other animals, they all showed values substantially above 3.18 nmol/l and pregnancy was confirmed.

Discriminatory values for skim milk progesterone are usually around 1 to 1.5 ng/ml (POPE et alii [13] 1976; GOWAN et alii [10], 1981; TAYLOR et alii [18], 1986). It has been suggested (REIMERS et alii [15], 1985) that samples below 1 ng/ml can be confidently diagnosed non-pregnant and those above 3 ng/ml as pregnant. However, 67.0% of the samples were within the range of 1 to 3 ng/ml and could only be considered questionable. This is not very satisfactory as a diagnostic procedure.

Three Gir cows with values below 3 nmol/l in skim milk were again considered non-pregnant and all negative diagnosis were correct. Nine cows with higher concentrations in day 23 were confirmed pregnant including one animal with a value of only 3.21 nmol/l. Milk or plasma could be used for pregnancy diagnosis but more results would be necessary to determine discriminatory values and questionable ranges for Brazilian conditions.

Estrus Detection Efficiency

(*) MATHER, E.C. - Personal communication.

Estrus detection in *Bos indicus* herds is known to be sometimes difficult, neverthless the efficiency in this study was quite high. In spite of the low number of cows utilized, the 83.0% detection indicate that a combination of visual observation and a teaser bull should provide accurate detection, which is sufficient for operation of an Artificial Insemination programme.

MADUREIRA, E.H.; BARNABE, R.C.; PINTO, P.A.; BARNABE, V.H.; MARTINS SOBRINHO, E.O. Níveis de progesterona determinados por radioimunoensaio em plasma sangüíneo e leite desnatado de vacas da raça Gir *(Bos indicus)*. Avaliações durante o ciclo estral. Diagnóstico precoce de gestação. *Braz. J. vet. Res. anim. Sci.*, São Paulo, *27*(2):247-253, 1990.

RESUMO: Foram estudados 17 ciclos estrais de 12 vacas da raça Gir, mantidas em regime extensivo de pastagens no Centro Intraunidade de Zootecnia e Indústrias Pecuárias "Fernando Costa", em Pirassununga, Estado de São Paulo. Para o estudo dos níveis de progesterona através de RIE, foram colhidas, 3 vezes por semana e aos 23 dias após a cobertura, amostras de leite e de plasma sangüíneo, totalizando, respectivamente, 137 e 138 amostras. Os resultados obtidos indicaram correlação entre amostras refrigeradas e congeladas de leite desnatado (r = 0,92), assim como entre leite desnatado e plasma sangüíneo (r = 0,63). Verificaram-se, ainda, variações dos níveis de progesterona durante o ciclo estral, com baixos valores na fase folicular (inferiores a 1,49 nmol/l de leite desnatado) e picos de 15,60 a 21,30 nmol/l no plasma e 5,91 a 11,37 nmol/l, no leite desnatado durante a fase luteínica. Todos os resultados de prenhez e não prenhez, baseados na análise de progesterona, utilizando níveis discriminatórios de 9,54 nmol/l no plasma e 3,18 nmol/l no leite, foram confirmados por palpação retal. As falhas de detecção de cio foram da ordem de 17,2% (24 cios observados em 29 ocorridos).

UNITERMOS: Progesterona; Leite desnatado; Plasma sangüíneo; Ciclo estral; Diagnóstico precoce de gestação; Radioimunoensaio; Prenhez, diagnóstico precoce; Bovinos, raça Zebu

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TABLE 1 - Progesterone levels (nmol/l) in [28] cooled or frozen skim milk samples. São Paulo, 1987.

milk condition / sample number	cooled	frozen
1	5.85	6.09
2	5.13	6.71
3	7.29	5.91
4	5.57	3.83
5	6.53	7.36
6	2.53	1.15
7	7.51	6.10
8	5.68	7.93
9	6.32	4.30
10	7.75	3.90
11	5.74	7.21
12	12.77	12.03
13	11.94	8.36
14	16.15	17.72
15	6.15	6.20
16	3.88	2.17
17	4.93	6.58
18	12.20	11.34
19	7.98	7.22
20	2.10	2.29
21	10.47	7.89
22	5.35	3.25
23	15.32	13.82
24	11.36	13.26
25	17.29	15.26
26	1.85	1.31
27	8.44	10.68
28	1.73	1.82
x	7.71 nmol/l	7.20 nmol/l
s	4.27 nmol/l	4.36 nmol/l

x = arithmetic mean
s = standard deviation

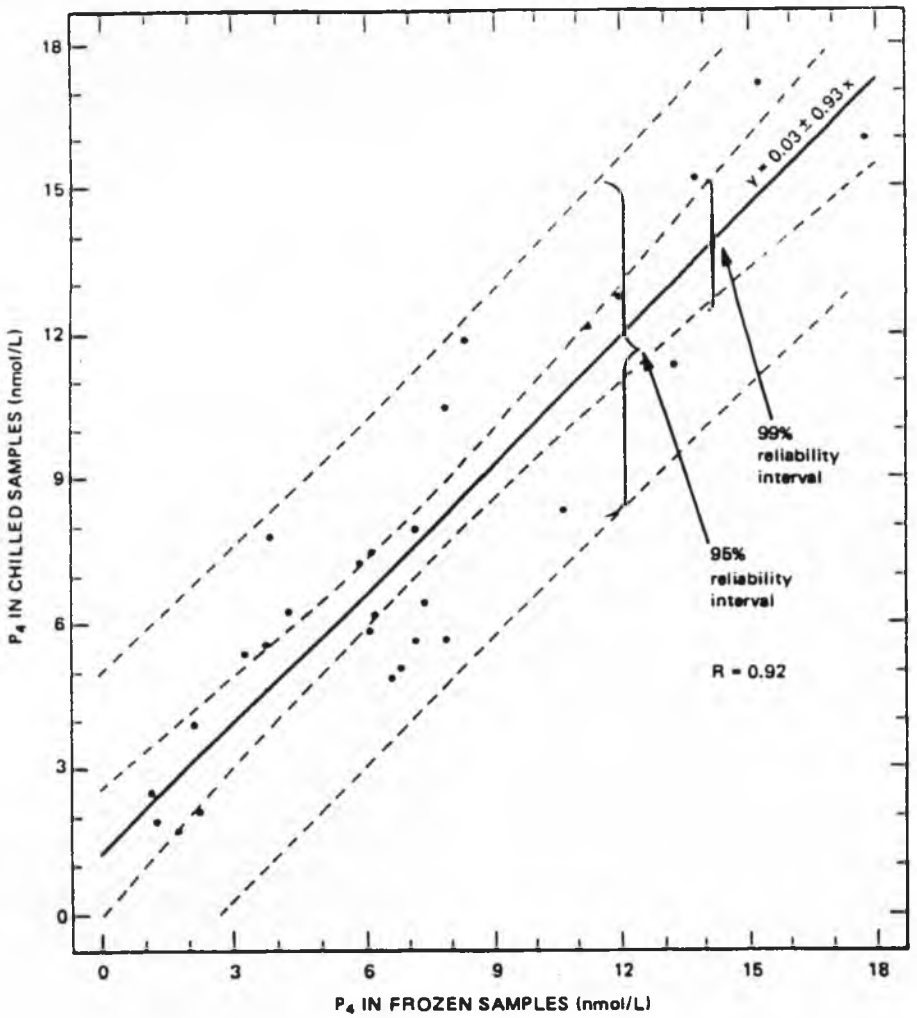

FIGURE 1— Correlation between progesterone in chilled (4 °C) and frozen (— 18 °C) skim milk samples.

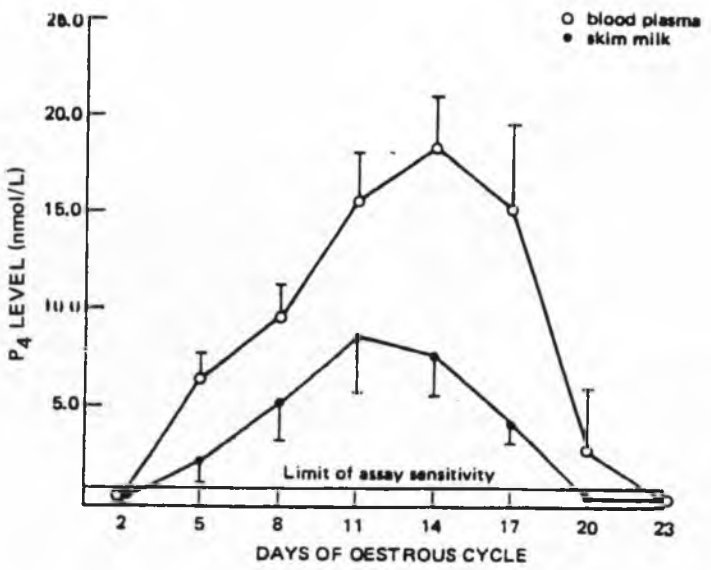

FIGURE 2 — Mean values (± SD) of progesterone levels in plasma and skim milk of Gir cows (**B. indicus**) during an oestrous cycle (São Paulo, 1987).