Progesterone profiles of postpartum dairy cows as an aid to diagnosis and treatment of reproductive disorders*

W.A. Butterfield and A.W. Lishman**

Department of Animal Science, University of Natal, P.O. Box 375, Pietermaritzburg 3200, Republic of South Africa

Milk progesterone levels were measured on a daily basis for 44 dairy cows from calving to conception in order to use the progesterone profiles in diagnosing and treating reproductive disorders related to the oestrous cycle. Some of the profiles could be used to identify reproductive abnormalities, for example, silent heats (13,6%), irregular oestrous cycles (2,2%), ovarian cysts (6,8%), and embryo mortality (15,2%). The degree to which such problems were overcome could also be monitored. The wide variation in progesterone profiles made it difficult to predict the outcome of insemination. It was concluded that repeat breeding could be due to several reasons, only some of which could be identified from progesterone profiles.

Daaglikse melkprogesteroonwaardes van 44 melkkoeie is bepaal vanaf kalwing tot besetting sodat progesteroon-profiele gebruik kon word om voortplantingsgebreke van die estrussiklus te diagnoseer en te behandel. Sekere van die profiele kon gebruik word om voortplantingsgebreke soos byvoorbeeld, stilbrons (13,6%), ongereelde bronstigheids-siklusse (2,2%), ovariumsiste (6,8%), en embriomortaliteit (15,2%) te identifiseer. Die mate waartoe sulke probleme oorkom is, kon ook gemonitor word. Weens die groot variasie in progesteroonprofiele was dit moeilik om die sukses van kunsmatige inseminasie te voorspel. Herhaalde onsuksesvolle inseminasie kon dus toegeskryf word aan verskeie oorsake waarvan net sommige deur middel van progesteroonprofiele identifiseerbaar was.

Keywords: Dairy cows, milk progesterone, profiles, reproductive activity.

* Part of an MSc. (Agric) thesis submitted by WAB to the University of Natal.
** To whom correspondence should be addressed.

Reproductive performance is one of the important factors determining the profitability of dairy herds (Bulman & Lamming, 1978). One measure of reproductive performance is the interval between parturition and conception. An extended calving interval is a major component of poor reproductive efficiency and is generally attributed to low fertility. However, it may also be the result of a managerial decision or deficiencies in the management programme.

The most common physiological manifestations of sub-fertility in dairy cows are lactation/postpartum anoestrus, silent heats, ovarian cysts, irregular oestrous cycles and embryo mortality (Lamming & Bulman, 1976; Bulman & Lamming, 1978; Foote, Oltenacu, Kummerfeld, Smith, Rieck & Braun, 1979). Taken individually, these fertility problems are of fairly low incidence. The incidence of some of these reproductive disorders may be increased by poor management, especially poor heat detection, and thus a technique to monitor such problems accurately is of obvious value.

Identification of progesterone in cows' milk (McCracken, 1963) led to the development of other methods for determining the concentration of progesterone in milk samples (Laing & Heap, 1971; Darling, Laing & Harkness, 1974). The rapid measurement of progesterone in milk using a semi-automated radioimmunoassay (RIA) is now a well established technique for pregnancy diagnosis in dairy cows (Booth & Holdsworth, 1976). Lamming & Bulman (1976) proposed that milk progesterone analysis may offer a convenient method of monitoring ovarian activity in large numbers of dairy cows to study abnormal patterns of progesterone secretion. They supported the view that measuring progesterone in samples taken twice weekly may provide a more rational basis for the treatment of sub-fertility in the

first eight weeks postpartum by differentiating between three possible conditions, viz. anoestrus, silent heats and persistent corpora lutea.

This study was initiated in an attempt to account for the 'repeat breeder' problem in dairy herds. Corrective treatment according to the diagnosis supported by progesterone profiles was also given.

Procedure

The two herds used in this study came from the Cedara and Ukulinga research farms. Initially, cows were selected on the basis of repeat breeding during the previous lactation (Group 1, n = 12). However, since the repeatability of the problem proved to be low, all cows in the herds were sampled daily over a period of 18 months as they came into lactation (Group 2, n = 19). Group 3 (n = 13) comprised cows which experienced reproductive problems (no observed oestrus for 50 days postpartum or no pregnancy after two inseminations) during the lactation under study. Sampling began as soon as the abnormality was detected. For Groups 1 and 2, sampling of milk was initiated 5—25 days postpartum.

Sampling was continued until the cow was diagnosed pregnant (rectal palpation) or was culled after three or more inseminations. Pregnant cows were removed from the study and replaced by cows which had recently calved. Cows which aborted or resorbed re-entered the investigation until pregnancy was confirmed. Oestrus was detected by twice daily observation, in addition to casual observation during daylight.

Milk samples were preserved using potassium dichromate (0,25%) and stored at −20°C until assay. The RIA method used to determine progesterone levels in milk was based on

the technique described by Holdsworth, Chaplin & Booth (1979) and modified by Butterfield (1986). Whole milk samples were used in the assay, which was performed directly, without ether extraction. A high-specificity antibody was used, as tested by immunological cross-reactivity studies with related steroids (Butterfield, 1986). The within- and between-assay coefficients of variation (CV's) of 6,7% and 13% respectively, were well below the minimum criteria of 20% for precision recommended by Abraham (1980).

Several cows in the study were treated with exogenous hormones in response to problems encountered during their open period.

Essentially, three types of abnormalities were detected and the corrective treatments were selected accordingly:

1. Undetected oestrus.

   Where cows were shown to be experiencing regular ovarian cycles, but oestrus was not detected, a single dose of prostaglandin $F_{2\alpha}$ (Estrumate, ICI) was administered, provided that a corpus luteum could be palpated. The cows were then inseminated at the first observed oestrus.

2. Inadequate luteal activity.

   Recurring short periods of luteal activity were treated by implanting synthetic progestogen (Synchromate B) two days after AI. Where this failed to result in pregnancy, pregnant mare serum (PMSG, Fostim) was administered during the subsequent cycle in an attempt to promote luteal function. Injections (600 IU) commenced two days after AI and were repeated every second day (Figure 2) for six days.

3. Ovarian cysts.

   Follicular cysts were treated by an injection of Receptal (5 ml), a synthetic releasing hormone for luteinizing and follicle stimulating hormone (Hoeschst). Persistent corpora lutea were not treated.

Results

No unambiguous cases of postpartum anoestrus (lack of cyclic activity for more than 50 days postpartum) occurred among the cows tested in this study, and therefore no corrective treatment for this type of abnormality was applied. Of the 44 cows studied, 29 (65,9%) were seen to be in oestrus within 50 days of calving. A further six cows (13,6%) underwent ovarian cycles, but were not observed in oestrus. The remaining nine cows (20,5%) exhibited first oestrus after 50 days postpartum. These cows formed part of Group 3, in

Table 1 Occurrence of 'silent' heats over a period of 18 months in two herds as determined from milk progesterone profiles

|	Cow herd	
	Cedara	Ukulinga
Total number of cows with profiles	17	21
Total number of observed heats	50	67
Number of silent heats	13 (26%)	20 (30%)
Number of cows exhibiting silent heats	10 (59%)	13 (62%)
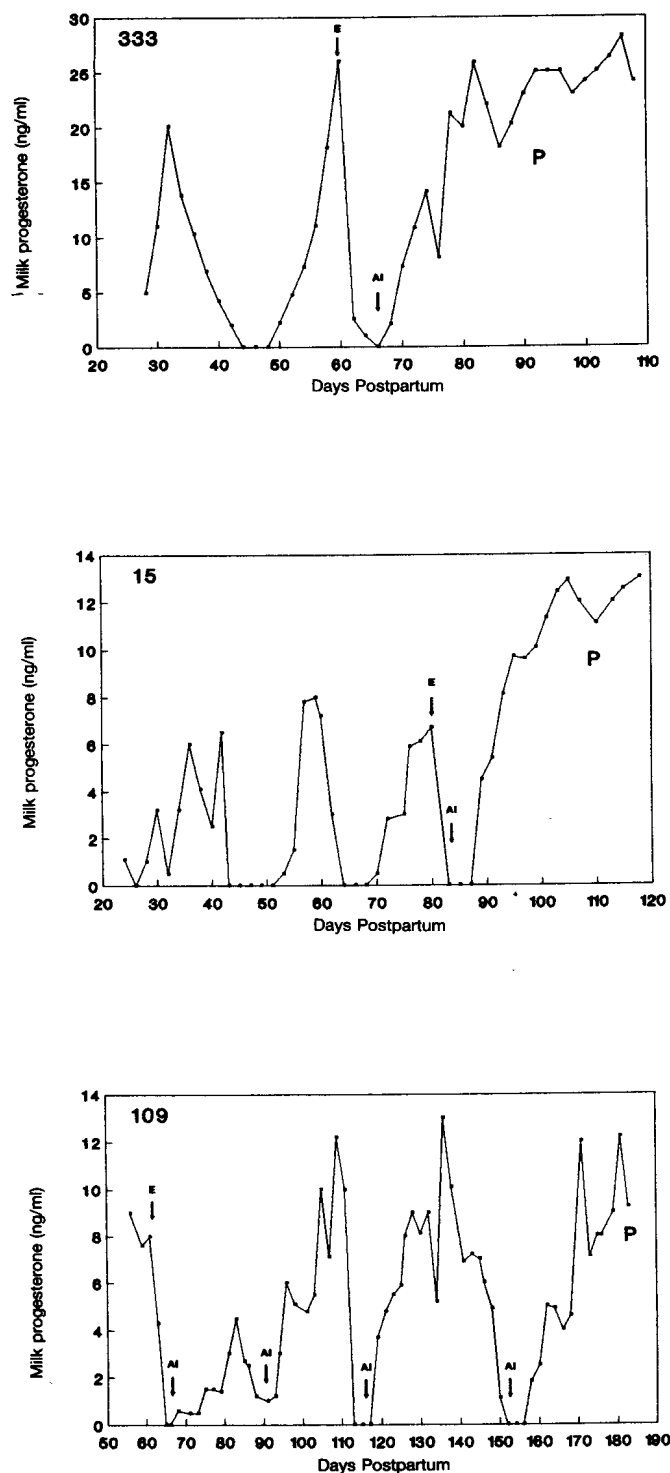
Figure 1 Progesterone profiles of cows treated with prostaglandin (Estrumate) where oestrus had not been observed. Cows 333 and 15 conceived at the induced oestrus whereas 109 exhibited poor luteal function in the cycle following prostaglandin administration. AI = insemination, E = Estrumate, P = pregnant.

which sampling was started later than 50 days postpartum. There were thus no progesterone profiles to indicate whether these cows had been cyclic or not prior to 50 days postpartum.

Milk progesterone profiles were used to determine the number of 'silent' or unobserved oestrous periods (Table 1).

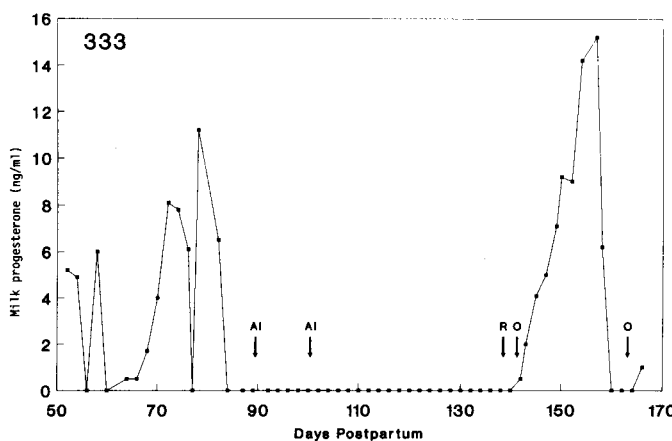Luteal phase progesterone levels were examined as a
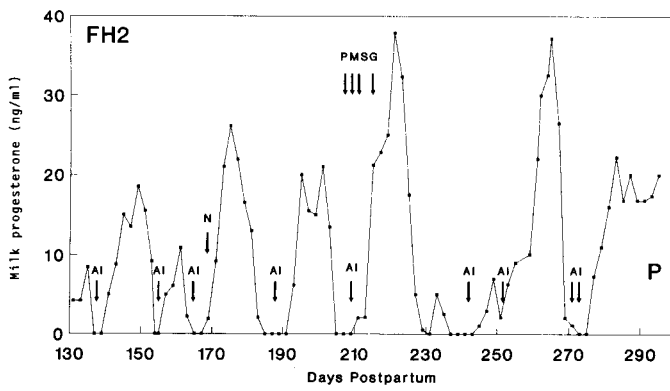
Figure 2 Milk progesterone profile of a cow exhibiting short oestrous cycles and implanted with progestogen (not removed) to simulate or PMSG to stimulate luteal function. AI = insemination, N = Norgestomet implant, PMSG = Fostim injection, P = pregnant.

possible cause of 'silent' heats. It was speculated that a relatively low peak progesterone level in the previous cycle might lead to the occurrence of 'silent' heat. The average peak progesterone levels before 'silent' and observed oestrous periods were 15,2 ng/ml and 16,5 ng/ml, respectively. The difference was not significant.

Two of the three cows treated for 'silent' heat, conceived (Figure 1). The third cow was inseminated at observed oestrus, but progesterone levels of the first ensuing cycle remained low. She conceived later.

Short oestrous cycles were exhibited by one cow (Figure 2). Milk progesterone concentrations continued to increase after norgestomet implantation. The luteal phase of the cycle was extended to 12 days and peak progesterone levels were increased relative to previous cycle levels. Since the cow did not conceive after treatment with progestogen, it was treated with PMSG in an attempt to stimulate the function of the corpus luteum and to promote pregnancy. Progesterone levels were increased following treatment (Figure 2), but after four injections a large cyst was palpated on the right ovary. Treatment was thus discontinued. The cyst was still present 14 days later, but progesterone levels had returned to basal levels. The cow subsequently conceived.

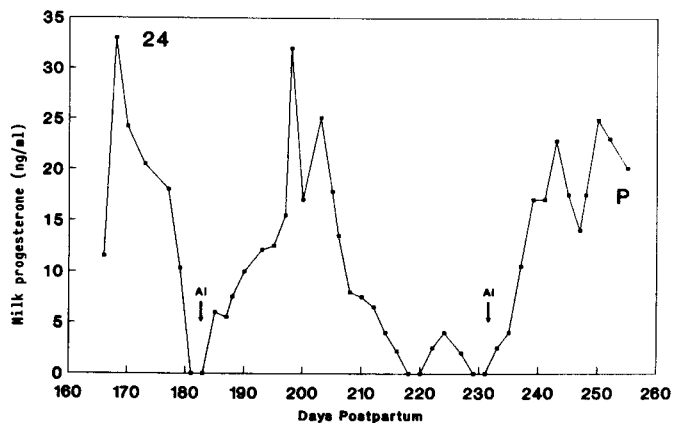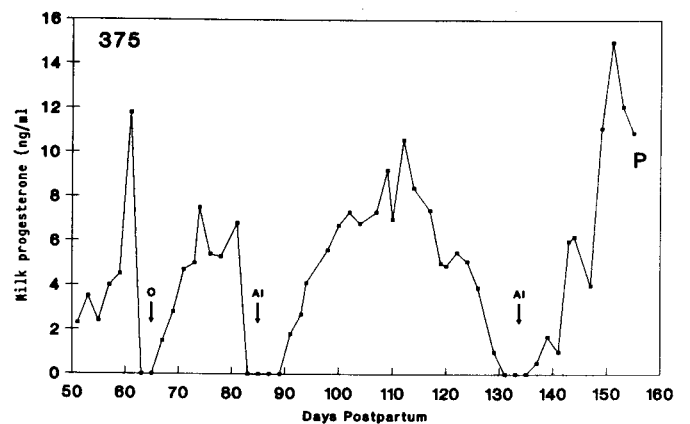A cow from the Cedara herd demonstrated an extended

period of basal progesterone concentrations (Figure 3). A follicular cyst was diagnosed, and this was confirmed by rectal palpation of the ovary. The cow was treated with Receptal (GnRH) and was seen to be in oestrus the following day. Milk progesterone levels indicated that cyclic ovarian activity commenced.

Embryo mortality was assumed to have occurred when oestrous cycles were longer than 28 days. The incidence of embryo mortality in this study, calculated from 79 oestrous cycles after insemination, was 15,2%.

Up to the return to oestrus, the progesterone profiles of two cows experiencing embryo mortality (Figure 4) were no different from those of cows undergoing normal pregnancies (Figure 5). It also appeared that embryo loss between 28 and 75 days after conception could not be attributed to inadequate luteal support.
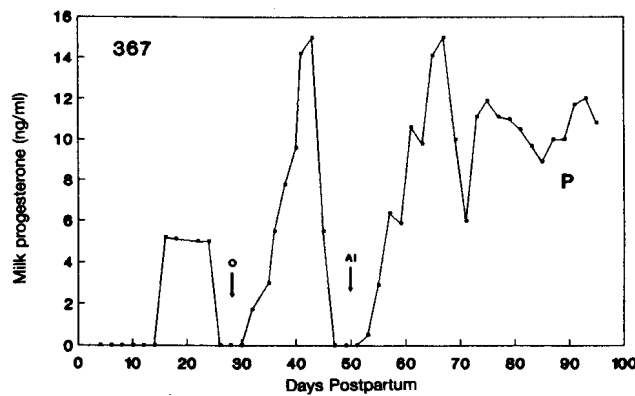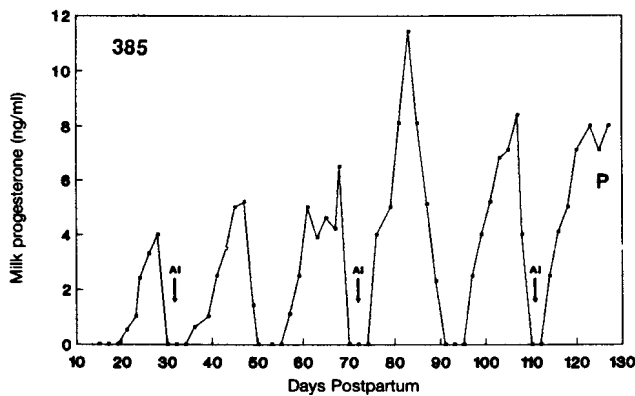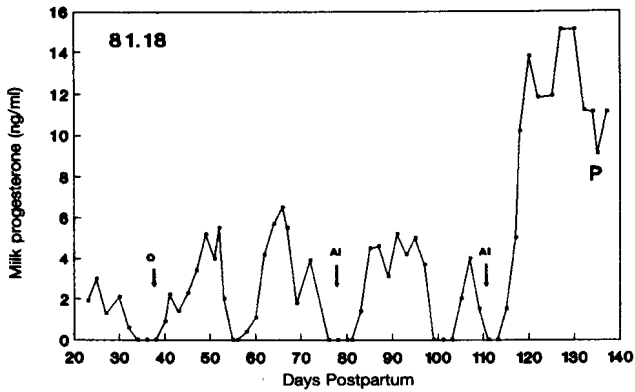
The progesterone profiles of some cows could not be categorized as specific fertility problems, but deviated from the normal situation. A normal profile was considered to be one in which the luteal phase, with progesterone levels greater than 5 ng/ml, lasts for 10—14 days. In order to compare such deviations, a progesterone profile of a cow classified as 'normal' is demonstrated in Figure 5. This cow experienced

Figure 3 Changes in progesterone secretion in a cow with a follicular cyst. AI = insemination, R = receptal, O = oestrus.

Figure 4 Extended duration of the luteal phase in cows where the embryo apparently died. AI = insemination, O = oestrus, P = pregnant.

the short-lived progesterone rise found in many cows at the time of cycle initiation. She ovulated, was seen to be in oestrus and had an oestrous cycle of normal duration. At the next oestrus the cow was inseminated, and she conceived. Using this as the normal situation, deviations from this pattern were assumed to indicate hormonal irregularities.

The reduced luteal phase progesterone levels of two cows, 81.18 and 385 (Figure 5), may have been the result of poor luteal support, although these two animals did cycle regularly. It was suspected that, without treatment, maintenance of pregnancy was unlikely. However, one cow conceived to an insemination performed after an extremely short-lived corpus luteum, and pregnancy was maintained. As expected, the other cow did not conceive.

The progesterone profiles shown in Figure 6 demonstrate an irregular pattern of progesterone secretion. The prognosis suggested that conception was unlikely to occur until regular cyclic activity had been initiated. This was proved incorrect in cow 284, as she conceived to her first insemination.

Discussion

Fertility problems which can be demonstrated using milk progesterone levels are those related to progesterone secretion from the corpus luteum during the oestrous cycle, viz. anoestrus, silent heats, ovarian cysts, irregular cycles and embryo mortality. Other problems, such as periovarian adhesions or oviduct obstructions cannot be detected using progesterone levels. Postpartum or lactation anoestrus has been defined as the absence of cyclic activity (milk proges-
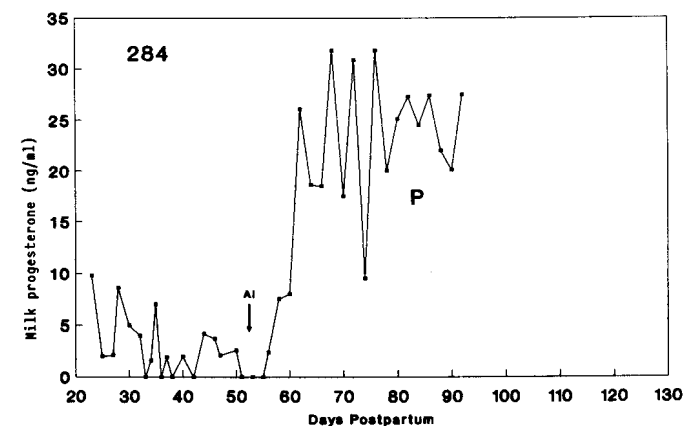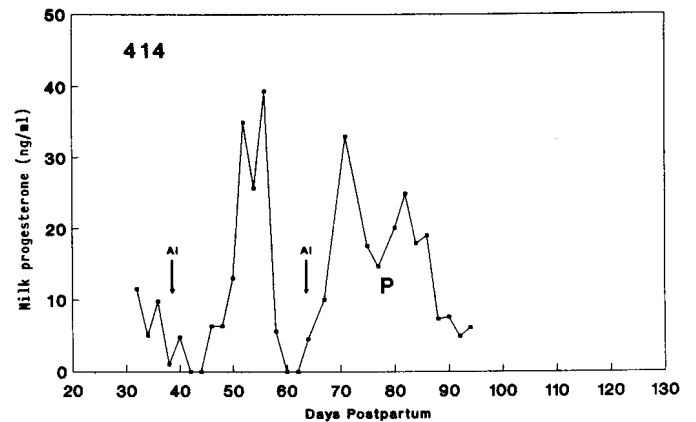
Figure 5 Reduced luteal phase progesterone levels immediately preceding successful (cow 81.18) or unsuccessful (cow 385) insemination compared to a 'normal' progesterone profile (cow 367) in the cycle prior to conception. AI = insemination, O = oestrus, P = pregnant.

Figure 6 Irregular patterns of progesterone secretion in two cows, one of which conceived only after a 'normal' cycle (cow 414) while cow 284 became pregnant in spite of the preceding atypical progesterone profile. AI = insemination, P = pregnant.

terone levels of 3 ng/ml or less) for more than 50 days after parturition (Bierschwal, Garverick, Martin, Youngquist, Cantley & Brown, 1975; Bulman & Lamming, 1978; Lamming & Bulman, 1976; Van der Wiel, Kalis & Shah, 1979; Peters & Lamming, 1984).

Cows may be incorrectly classified as non-cyclical if a 10-day blood sampling interval is used. This may arise owing to short luteal phases (Mather, Camper, Vahdat, Whitmore & Gustafsson, 1978), long periods of relatively low progesterone levels around ovulation (Jackson, Johnson, Bulman & Holdsworth, 1979) and in cases where the first postpartum ovulation occurs a few days before the second milk sample. During this survey, milk samples were collected daily, but analyses were done on every second sample. There were thus no problems in classification of cows.

If it is assumed that the nine cows that exhibited first oestrus after 50 days postpartum were acyclic during this time, the incidence of anoestrus in the study was 20,5%. This is higher than reported figures, which range from 5% (Pope & Swinburne, 1980) to 12% (Van der Wiel et al., 1979).

Another symptom typical of problem cows is silent heat. This has been defined as oestrous symptoms that are not observable by the herdsman, but where milk progesterone measurements reveal a normal cyclic pattern (Claus, Karg, Zwiauer, Von Butler, Pirchner & Rattenberger, 1983). Studies have shown that at least 50% of all cows have one silent heat before showing the first behavioural oestrus (King, Hurnick & Robertson, 1976; Schams, Schallenberger, Menzer, Stangel, Zottmeier, Hoffmann & Karg, 1978). The number of observed oestrous periods increases thereafter and the incidence of silent heats has been found to be negligible by the end of the fourth month after parturition (Zemjanis, 1980). However, silent heat has been found to be present in at least 32% of all normal postpartum cycles (Claus et al., 1983), and shown by 48% of cows in a herd (Van der Wiel et al., 1979).

In herds observed continuously, it has been found that silent heats are not a problem once cyclic ovarian activity had been initiated (King et al., 1976). Zemjanis (1980) maintained that the incidence of silent heats could be reduced to a minimum by increasing the number of oestrous observations each day. This points towards deficiencies in oestrous detection routines, rather than deficiencies in the reproductive hormones.

In this study, 28% of all normal cycles were not detected by observations for oestrus, with 60,5% of cows showing silent heat. The possibility thus exists that some of the ovulations, not accompanied by overt oestrus in this study, were unobserved due to some flaw in the oestrous detection routine rather than a silent ovulation. The milk progesterone assay can thus be used to monitor, in retrospect, the efficiency of oestrous detection.

It was evident from milk progesterone profiles that progesterone levels prior to oestrus varied widely between individual cows, and between cycles for a single cow. It was thus concluded that such profiles could not be used to indicate conditions under which silent heat is likely to occur.

The occurrence of ovarian cysts is another factor influencing the number of problem cows. There are three types of cyst. Follicular and luteal cysts are anovulatory, the former characterized by low and the latter by high progesterone levels (Kesler, Elmore, Youngquist, Garverick & Bierschwal, 1977). Persistent corpora lutea are non-pathological and consist of a corpus luteum containing a cavity with a diameter of >10 mm (Donaldson & Hansel, 1968). The incidence of ovarian cysts in this investigation was 6,8%, which was similar to the reported range of 2% (Lamming & Bulman, 1976) to 6% (Van der Wiel et al., 1979).

In this study, milk progesterone analysis proved to be an invaluable aid to:

(a) early identification of the presence of a cyst,
(b) differential diagnosis of cysts, and
(c) application of appropriate treatment.

Cows experiencing embryo mortality are also classified as problem cows. Embryo mortality refers to fertility losses during the period, lasting up to approximately 45 days after insemination (Ayalon, 1978).

The frequency of embryo mortality in this study (15,2%) was similar to those reported in other studies (Kummerfeld, Oltenacu & Foote, 1978; Claus et al., 1983) where milk progesterone analysis was used. Milk progesterone levels provide an estimate of embryo mortality which is far more accurate than that obtained using delayed returns to oestrus. Using the latter method of estimation, the incidence of embryo mortality in these cows would have been 29,5%. Thus, a substantial portion of the estimate of embryo mortality by delayed returns is bias due to missed oestrous periods (Kummerfeld et al., 1978).

It was not possible to demonstrate the cause of embryo death from the progesterone profiles of cows experiencing embryo mortality, and milk progesterone levels were of no use in predicting the cows in which embryo mortality might occur. Similar results were reported by Bulman & Lamming (1979).

Erb, Garverick, Randel, Brown & Callahan (1976) found that infertile inseminations were often accompanied by asynchronies in the hormonal profile. However, Bulman & Lamming (1978) concluded that failure to conceive could not, in general, be related to differences in milk progesterone secretion patterns. Some infertile cows had irregular profiles which identified six categories of reproductive abnormalities, but progesterone profiles alone did not differentiate the cows which conceived from those which had embryo mortality. They concluded that only some of the reasons for repeat breeding could be identified by variations in endocrine activity. The findings in the present study support these conclusions, since, although a normal pattern of progesterone secretion could be identified, the profiles varied considerably between and within cows. This made it extremely difficult to predict the outcome of insemination.

The potential value of monitoring progesterone levels in postpartum dairy cows as an aid to the assessment of reproductive activity in dairy herds, has been demonstrated (Bulman & Lamming, 1978) and is confirmed by this study. The present findings support the conclusion that progesterone assays cannot be used in isolation, but can provide a more precise diagnosis of certain factors responsible for sub-fertility.

Acknowledgements

References

ABRAHAM, G.E., 1980. Reliability criteria for steroid radioimmunoassay. In: Radioimmunoassay of steroid hormones. Ed. Gupta, D., Verlag. Chemie, Weinheim. p. 9.

AYALON, N., 1978. A review of embryo mortality in cattle. *J. Reprod. Fert.* 54, 483.

BIERSCHWAL, C.J., GARVERICK, H.A., MARTIN, C.E., YOUNGQUIST, R.S., CANTLEY, T.C. & BROWN, M.D., 1975. Clinical response of dairy cows with ovarian cysts to GnRH. *J. Anim. Sci.* 41, 1660.

BOOTH, J.M. & HOLDSWORTH, R.J., 1976. The establishment and operation of a central laboratory for pregnancy testing in cows. *Br. Vet. J.* 132, 518.

BULMAN, D.C. & LAMMING, G.E., 1978. Milk progesterone levels in relation to conception, repeat breeding and factors affecting acyclicity in dairy cows. *J. Reprod. Fert.* 54, 447.

BULMAN, D.C. & LAMMING, G.E., 1979. The use of milk progesterone analysis in the study of oestrous detection, herd fertility and embryonic mortality in dairy cows. *Br. Vet. J.* 135, 559.

BUTTERFIELD, W.A., 1986. The progesterone assay of milk as an indicator of the reproductive status of the dairy herd. M.Sc.(Agric) thesis, University of Natal.

CLAUS, R., KARG, H., ZWIAUER, D., VON BUTLER, I., PIRCHNER, F. & RATTENBERGER, E., 1983. Analysis of factors influencing reproductive performance of the dairy cow by progesterone assay in milk fat. *Br. Vet. J.* 139, 29.

DARLING, J.A.B., LAING, A.H. & HARKNESS, R.A., 1974. A survey of the steroids in cows' milk. *J. Endocr.* 62, 291.

DONALDSON, L.E. & HANSEL, W., 1968. Cystic corpora lutea and normal and cystic Graafian follicles in the cow. *Aust. Vet. J.* 44, 304.

ERB, R.E., GARVERICK, H.A., RANDEL, R.D., BROWN, B.L. & CALLAHAN, C.J., 1976. Profiles of reproductive hormones associated with fertile and non-fertile inseminations of dairy cows. *Theriogenology*, 5, 227.

FOOTE, R.H., OLTENACU, E.A.B., KUMMERFELD, H.L., SMITH, R.D., RIECK, P.M. & BRAUN, R.K., 1979. Milk progesterone as a diagnostic aid. *Br. Vet. J.* 135, 550.

JACKSON, P.S., JOHNSON, C.T., BULMAN, D.C. & HOLDSWORTH, R.J., 1979. A study of cloprostenol-induced oestrus and spontaneous oestrus by means of the milk progesterone assay. *Br. Vet. J.* 135, 578.

HOLDSWORTH, R.J., CHAPLIN, V.M. & BOOTH, J.M., 1979. Radioammunoassay of progesterone in milk: development of techniques for large-scale use as a test of pregnancy. *Br. Vet. J.* 135, 470.

KESLER, D.J., ELMORE, R.G., YOUNGQUIST, R.S., GARVERICK, H.A. & BIERSCHWAL, C.J., 1977. Ovarian morphology in dairy cows with ovarian cysts following treatment with GnRH. *J. Anim. Sci.* 45, (Suppl. 1) 176 (Abstr.).

KING, G.J., HURNICK, J.F. & ROBERTSON, H.A., 1976. Ovarian functions and estrus in dairy cows during early lactation. *J. Anim. Sci.* 42, 688.

KUMMERFELD, H.L., OLTENACU, E.A.B. & FOOTE, R.H., 1978. Embryonic mortality in dairy cows estimated by non-returns to service, oestrus, and cyclic milk progesterone patterns. *J. Dairy Sci.* 61, 1773.

LAING, J.A. & HEAP, R.B., 1971. The concentration of progesterone in the milk of cows during the reproductive cycle. *Br. Vet. J.* 127, xix.

LAMMING, G.E. & BULMAN, D.C., 1976. The use of milk progesterone radioimmunoassay in the diagnosis and treatment of subfertility in dairy cows. *Br. Vet. J.* 132, 507.

MATHER, E.C., CAMPER, P.M., VAHDAT, F., WHITMORE, H.L. & GUSTAFSSON, B.G., 1978. Assessment of ovarian activity in the postpartum dairy cow by use of a milk progesterone assay. *Theriogenology* 10, 119.

McCRACKEN, J.A., 1963. The distribution of progesterone in body fluids and tissues of the dairy cow. Ph.D. thesis, University of Glasglow.

PETERS, A.R. & LAMMING, G.E., 1984. Reproductive activity of the cow in the post partum period. 2. Endocrine patterns and induction of ovulation. *Br. Vet. J.* 140, 269.

POPE, G.S. & SWINBURNE, J.K., 1980. Reviews of the progress of Dairy Science: Hormones in milk; their physiological significance and value as diagnostic aids. *J. Dairy Res.* 47, 427.

SCHAMS, D., SCHALLENBERGER, E., MENZER, C., STANGEL, J., ZOTTMEIER, K., HOFFMANN, B. & KARG, H., 1978. Profiles of LH, FSH and progesterone in postpartum dairy cows and their relationship to the commencement of cyclic functions. *Theriogenology* 10, 453.

VAN DER WIEL, D.F.M., KALIS, C.H.J. & SHAH, S.N.H., 1979. Combined use of milk progesterone profiles, clinical examination and oestrus observation for the study of fertility in the postpartum period of dairy cows. *Br. Vet. J.* 135, 568.

ZEMJANIS, R., 1980. Anoestrus in cattle. In: Current therapy in Theriogenology. Ed. Morrow, D.A., W.B. Saunders, Philadelphia.