THE USE OF MILK PROGESTERONE RADIOIMMUNOASSAY TO ASSESS FERTILITY IN THE POST-PARTUM PERIOD OF DAIRY COWS

By

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ABSTRACT

The pattern of post-partum and ovarian activity was monitored in two herds through the use of radioimmunoassay of progesterone in post-milking strippings. The University of British Columbia, South Campus (UBC) herd was sampled on a twice weekly basis, while the Agriculture Canada Research Station, Agassiz herd was sampled every second day. Sampling began approximately six days post-partum and continued up to 60 days post-conception.

The estrous cycle was classified into phases based on the concentration of progesterone in the milk samples. Progesterone concentration immediately post-partum and for a varying length of time thereafter remained at basal levels. This was classified as Phase 0 and represented the stage of quiescence in the ovaries post-partum. Phase 1 of the estrous cycle represented the follicular stage when the concentration of progesterone was low. Phase 2 represented the stage when the corpus luteum was developing and the concentration of progesterone was rising. Phase 3, the fully active corpus luteum stage, was when the concentration was highest, while Phase 4 represented the regressing corpus luteum stage when the concentration was falling.

The stage of quiescence, from parturition to the initiation of luteal activity was found to be 21.43 ± 11.84 days for 54 animals in the UBC herd and 19.81 ± 8.85 days for 127 animals in the Agassiz herd. Retained placenta increased the duration of quiescence to 25.62 ± 12.41 days for the 12.6% or 16 animals having the condition in the Agassiz

herd. The 9.3% or five animals having retained placenta in the UBC herd had a much shorter stage of quiescence of 10.80 ± 6.42 days.

Two distinct types of first cycles were found, based on the mean concentration of progesterone and the number of days in the period. A normal first cycle exhibited more luteal activity during Phase 3, and remained in this Phase longer than did the shortened first cycle. This may be associated with follicle luteinization in the shortened first cycle rather than true ovulation as in the normal first cycle.

The increased frequency of sampling of the Agassiz herd tended to give a more accurate classification of the phases of the cycle as defined, with more Phase 4 samples being identified. Also days in Phase 1 and Phase 2 for both Type 1 (shortened first cycle), and Type 2 (normal first cycle), were approximately half of those observed from the UBC herd.

Standard curves of progesterone concentration vs. time for "normal" cycles of various lengths with standard deviations were derived from the pooling of all cycles classed as normal first cycles and normal cycles without a breeding. These "normal" cycles showed that as the cycle length increased, so did time spent during Phase 1 when the concentration of progesterone remained below 4 ng/ml milk.

Progesterone profiles of various animals are included, illustrating the wide variety of profiles found. These include profiles of short stages of quiescence, long stages of quiescence, short cycles, long cycles as well as some abnormalities observed.

When used as a test for early detection of pregnancy, a single sample from days 21 to 24 for the UBC herd showed accuracies of 100% in

determining non-pregnancy and 86% in determining pregnancy. Test results for days 21 and 22 for the Agassiz herd were again 100% accurate for determining non-pregnancy and 90.5% accurate in determining pregnancy. The accuracy of the non-pregnant determination can be increased by including a sample on day of insemination and eliminating those animals inseminated at an obviously incorrect time. With the two sample test on the combined UBC data the acuracy of the pregnant diagnosis increased to 91.5%. On the combined Agassiz data the two sample test increased the accuracy of the pregnant diagnosis to 93.1%.

The use of progesterone pregnancy testing offers a considerable saving in time in identifying those animals not conceiving to insemination and a reasonably accurate means of early detection of pregnancy.

The average number of days from conception to positive palpation was 51.02 ± 13.19 days for 97 animals in the Agassiz herd and 60.43 ± 23.59 days for the 42 animals in the UBC herd.

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INTRODUCTION

Milk is the one food for which there seems to be no adequate substitute. It constitutes almost the entire diet for the young of all mammals (Hughes and Bennion, 1972). The dairy cow is thought by many to be the mother of mankind. "We rob her of her children that we may rob her of her milk" (Fussel, 1966).

Many authors have indicated that failure to breed is an important reason for discarding animals from herds, particularly in dairy cattle (Dawson, 1977; Melrose, 1979; Foote, 1979). Foote (1970) reported sterility as the second most frequent cause of culling dairy cows from New York State herds. Lineweaver and Spessard (1975) reported that reproductive inefficiency alone accounted for an estimated $540 million loss in the United States.

Reproductive failure in dairy cattle causes economic loss directly as a result of its effect on yearly milk production and on surplus calves for sale (Louca and Legates, 1968), and indirectly because the potential selection differential is reduced with fewer replacements (Foote, 1970). While culling for low yield is part of most breeders genetic improvement programme, cows culled for reproductive problems decrease the opportunity to select for yield and, therefore, deter genetic improvement. As well, by increasing the days open and days dry, the generation interval is increased and the potential rate of genetic improvement is decreased over a given period of time (Foley et al., 1972).

The optimum calving interval for the dairy cow has been the subject of much study in the past. Early conception and short dry periods are reported to have depressing effects on lactation and milk yields (Smith and Leagates, 1962). Speiker and Meadows (1967) recommended twelve month calving intervals. Louca and Legates (1968) found during the first lactation for each additional day open there was a gain of 1.16 kg. milk/day and a decline of 3.58 and 3.68 kg. for the subsequent lactations. Therefore, they recommended that a 13 month calving interval would be optimal for heifers, while 12 months for second and later calvings was optimal. Higher producing, more persistent cows, however, may not suffer the production losses observed in average cows, when the calving interval is extended to 13 or 14 months (Foley et al., 1972). Schneider et al. (1981) found that cows with an average calving interval of 13 months produced more fat-corrected milk in 305 day lactation and in the first 150 days of the subsequent lactation than cows with an average calving interval of 12 months.

Dawson (1977) reported on a study of the reproductive potential in female cattle discarded as infertile, in which he found that in fact many of the animals were fertile. These animals had simply reached a point in days open, when it was no longer economical for the producer to continue to attempt to get them bred (Foote, 1970; Bulman and Lamming, 1979 and Dawson, 1977). The results of Dawson (1977) suggest that infertility of immature cattle on farms is often only temporary. As skeletal growth is not complete in cows until 4-5 years of age, Dawson (1977) speculated that early first calving could be followed by a

suspension of reproductive function, until the metabolic demands for growth had been satisfied. When mature dairy cows fail to breed, the infertility is more likely to be permanent, and is often associated with the activity of pathogens (Dawson, 1977).

Karg (1981) gave the following description of fertility:

> "Fertility is a multifactorial phenomenon where the converses (given in parenthesis) of each of the following events are major factors in infertility: establishment of cyclicity during puberty (acyclia); oestrus, insemination, ovulation (silent or unrecognized heat, false timing of AI, delayed ovulation); fertilization, development of the conceptus, shift to maternal conditions, pregnancy (embryonic death, abortion); parturition, delivery of the placenta, uterine involution (stillbirth, dystocia, retained membranes, uterine infections); and re-establishment of cyclicity post partum (acyclia, cysts, etc.). Modulating factors are breed, nutrition, housing conditions, season, age, milk yield, suckling, resting time post-partum, regulating disorders, infections, etc."

The discovery that progesterone occurs in milk and, that its concentration reflected the variations of the estrous cycle and the plateau of pregnancy, and later, the development of a simple and rapid radioimmunoassay for progesterone, has provided a means by which we can assess fertility in the dairy cow.

The present study illustrates the use of progesterone radioimmunoassay of sequential milk samples, a non-invasive technique, to assess fertility in the post-partum cow.

The objective of this study was to develop a method of examining and classifying the profiles of progesterone concentration in milk, generated by twice a week sampling, and every other day sampling, post-partum to post-conception, of cows located in two herds.

To this end, the cycle was divided into a series of phases, and based on the duration and level of progesterone in each, the cycles were categorized. A secondary objective of this study was to develop a standard curve for "normal" cycles, with progesterone concentration means and standard deviations for each sampling date.

The study also included an examination of the reliability of using milk progesterone sampling as a method of early determination of pregnancy.

LITERATURE REVIEW

The main purpose of this paper is to review the use of information obtained by milk progesterone radioimmunoasay to assess the fertility in the post-partum period of dairy cows. However, as Karg (1981) states, "Fertility is a multifactorial phenomenon." It seems reasonable, therefore, to review some of the many factors influencing the fertility of a dairy cattle population. This will be followed by a review of the early work with milk progesterone, and some of the ways in which it may be used to provide a better understanding of fertility in dairy cattle.

A. Factors Influencing Fertility

1. The Bull

While this review deals primarily with reproduction from the female side, some mention should be made of the male. When natural service is used, it is often the bull that is the cause of failure of the cow to become pregnant. The bull should be thoroughly examined by a competent veterinarian prior to inclusion in any breeding programme.

When AI is used, and semen is obtained from one of the many stud services, then one can feel confident that the bull has been thoroughly examined, and meets the minimum standards set out by the breed organizations. Those bulls which fail to fertilize or do so inadequately are eliminated. However, a difference of 15% with regard to conception rate after the first insemination, between the bull displaying the best, and the bull showing the poorest fertilizing

capacity, is a common phenomenon in AI centres of average size (De Kruif, 1978).

2. Handling Frozen Bovine Semen In the Field

Most poor fertility results with frozen semen are due to improper handling or deposition of the semen, or both, by the inseminator (Pickett and Berndtson, 1980).

Successful storage of frozen semen requires a storage temperature of -130°C or lower at all times, for maximum reproductive efficiency. This requirement is met with liquid nitrogen which has a temperature of -196°C. Because of the relatively large surface area, and the relatively small volume of extended semen contained in most straws, the straws are conducive to rapid heat exchange. Care must be taken to make any transfer of semen from one storage unit to another as quickly as possible. Any time the temperature of the semen rises above -130°C, damage can be expected. Such damage, due to elevated temperature, is additive.

Most AI centres monitor the first service conception rates of each of their inseminators and there are marked differences between inseminators and within inseminators over time (De Kruif, 1978). Salisbury and Flerchinger (1967) studied the effects of aging in spermatozoa and found that the fertilizing capacity of the spermatozoa decreased, and an associated increase in prenatal death of the developing zygotes was noted, as the time from collection to insemination increased.

3. The Cow

Swensson and Andersson (1980) reported on a paper by R.K. Bar-Anan et al. (1979) who stated that they found an indication that a genetic component in the ovum or uterus could cause death of the embryo at implantation, which was expressed only as a delay of the following heat period.

It is concluded by nearly all investigators that the pregnancy rate is reduced in those animals which have calved for the first time, with the majority of these investigators attributing the reduction in conception rates to increased problems at the time of parturition and during puerperium (De Kruif, 1978). De Kruif (1978) observed a 5% increase in pregnancy rate between primiparous and secundiparous cows. This increase continued up to four years of age, and according to Foley et al., (1972) remains constant from four to six years and then gradually decreased with advancing age. This decrease was noted by De Kruif (1978) as well.

Gwasdauskas et al. (1975) reported first service conception rates for various ages of cows as follows: heifers 47.6%, young cows 42.7% and older cows 31.9%. However, age is not a major cause of infertility in dairy cattle, as most of the animals in a production herd will be culled for one reason or another before they reach old age. What is more important is size of the heifer at first breeding.

Heifers normally reach puberty at six to ten months of age (Noakes, 1979), depending upon the breed. As noted earlier, much of the

decrease in conception rates following first calving can be attributed to problems associated with parturition, as the first calf heifers have greater difficulty than older and larger cows. Therefore, it is of great importance that the heifer achieve sufficient size prior to first breeding, to minimize the risks of dystocia. Delaying the breeding of normally fed heifers who have reached the recommended size, beyond 15 months of age, reduces the total lifetime production, and, if the delay is continued up to three years of age, the heifer may develop reproductive disorders.

Table 1

Recommended Body Weight of Heifers

	At First Breeding		Post Calving	
	Pounds	Kg	Pounds	Kg
Holstein	750	335	1100	500
Brown Swiss	750	335	1100	500
Ayrshire	600	270	1000	450
Guernseys	550	250	950	425
Jerseys	500	225	850	380
	Folley et al. 1972		Hafs & Boyd 1973	

4. Estrus Detection

Estrus detection is a major problem for all dairymen using artificial insemination (Whitmore, 1980).

To maintain even production levels throughout the year, dairymen must have cows calving each month, consequently, poor estrus detection may give rise to a wide variety of fertility management problems within

the herd, such as increased interval between parturition and first insemination, which in turn leads to a lengthened period from parturition to conception (Bozworth et al., 1972; Barr, 1975). Due to poor estrus detection cows previously inseminated which have not conceived, may pass unnoticed. This results in an unduly long interval between inseminations, again, lengthening the period of time between parturition and conception or days open.

The signs of estrus can be divided into primary and secondary behavioral traits (Holstein Friesian Journal, 1979). The primary and most important sign of true estrus is the standing heat, when the animal is standing firm, with all four legs braced, while being mounted by another animal. Secondary signs of estrus may be mounting of the cows, swollen vulva, hair over rump ruffled or rubbed off, mucus discharge, hyperactivity, i.e., walking along fence lines, standing while others are lying down, bellowing, clear mucus discharge from vulva, reduced intake and reduced production, and these may be exhibited before, during and after true estrus. Cows missed, may be noted by metestrus bleeding (Foote, 1975, Foley et al., 1972, Holstein Friesian Journal, Sept. 1979, Whitmore, 1980).

Animals submitted for A.I. on the basis of the primary sign usually have a high pregnancy rate, while those submitted solely on the basis of secondary signs, and those animals which are wrongly submitted for one reason or another (inadequate identification) have a much lower pregnancy rate and usually return to estrus (often irregularly) increasing the repeat breeder problem, and increasing the services per conception ratio (Whitmore, 1980).

De Kruif (1978) reported on papers by Roberts (1971) and Gunnink (1973) that endometritis may occur incidentally in these animals because the resistance of the uterus to bacteria is lowered during the luteal phase. This could be one of the reasons for the increase in repeat breeder problem cows in the group of animals mentioned by Whitmore (1980).

"Silent estrus" and anestrus are often the dairyman's favourite excuses for what in reality are missed heats. Zemjanis (1980) stated, "silent estrus or quiet ovulation, is physiological during the immediate post-parturient period, in both dairy and beef cattle. Its significance as the cause of anestrus at the time of planned breeding is highly questionable." De Kruif (1978) concluded, "it is apparent from several studies that almost all cows in a herd show signs of estrus but that these are inadequately observed, particularly in large herds." (Hurnik, et al., 1975, King et al., 1975 and King et al., 1976.) Continuous observation was found to be 100% effective in detecting estrus in a large dairy herd while casual observation by the herdsman detected only 56% of the same animals (Williamson et al., 1972). It can therefore be concluded that silent heat is a matter of management rather than of cows, and the incidence of silent estrus can be reduced to a minimum by increasing the number of heat observation periods.

There are several types of heat detection aids which have been used to augment direct observation. These include, heat mount detectors, "marker bulls" which have been surgically prepared to prevent

mating and fitted with a halter containing a marking device, testosterone treated cows and steers similarly fitted with a marking device, marking all cows with chalk and observing for those with chalk rubbed off, electrical measurement of vaginal mucus (Gartland et al., 1976), use of pedometers to measure activity of the cow (Kiddy, 1977), and the use of video equipment to assist observation (Foote, 1975).

It must be rememberd that these are just aids and it is still the requirement of management that time be spent to properly identify those animals in estrus for A.I.

5. Timing of Insemination

5.1 During Estrus

It is particularly important in artificial insemination that the cow be bred at the correct time during the estrus period. When natural breeding is used, the cow normally will only allow the bull to mount when it is most opportune. However, when A.I. is used, it is up to management to determine when to inseminate the cow.

Fertility is not uniformly distributed throughout estrus. When inseminated during the early part of estrus, fewer cows will conceive, particularly when the semen is of indifferent or inferior quality. Inseminations after ovulation has occurred will also result in lower pregnancy rates (Trimberger and Davis, 1943; Boyd, 1970; Deas, 1970; Hafs and Boyd, 1973; de Kruif, 1978). Lodge (1976) reported that aged ova can result in lowered fertility, or, problems associated with the early development of the fertilized ova.

Estrus in the dairy cow lasts from 12 - 18 hours while ovulation occurs from 4 - 16 hours after estrus (Mather and Rushmer, 1979). The ova remains fertile for only approximately 6 - 10 hours and spermatozoa may require up to 6 hours within the cow's reproductive tract to develop the capacity to fertilize the ova (Hafs and Boyd, 1973). Therefore, the optimal breeding time is from 10 - 16 hours after the onset of estrus (Mather and Rushmer, 1979) or between the middle and the end of the period of estrus (Trimberger and Davis, 1943; Bane, 1964; Swensson and Andersson, 1980). With once a day heat detection an animal seen in standing heat could be beginning estrus or ending, complicating the decision of correct timing of insemination.

General recommendations by Hafs and Boyd (1973) and of many A.I. organizations are:

1. Cows first noticed in standing heat in the morning should be bred that afternoon;

2. Cows first noticed in standing heat in the afternoon should be bred before noon the following day.

5.2 Post-Partum

The timing of rebreeding post-partum has been the focus of many studies over the years. Here again we have a management decision. Are we concerned with high conception to first service, or the shortest interval from calving to conception. Is it maximal lactational yield that is the main concern, or is it maximum lifetime production? The

desirability of a twelve month calving interval has been discussed (see introduction). To achieve this interval, cows must conceive on the average by approximately 85 days post-partum.

Ayalon, et al., (1971) suggested that it was necessary to begin breeding prior to 60 days post-partum due to the variability in the date of appearance of heat, and waiting until 60 days post-partum would result in a much longer interval. Lowered conception rates have been reported in cows inseminated prior to 50 days post-partum (Shannon, et al., 1952; Touchberry et al., 1959; Whitmore et al., 1974, Britt, 1975; and Whitmore, 1980).

Schneider, et al., (1981), comparing the effects of early and late breeding, found that in the group bred early, at first heat after 50 days post-partum, conception occurred by 88 days post-partum, and required 1.5 services per conception. Breeding at first heat after 80 days post-partum delayed conception until 121 days, and decreased fertility, requiring 1.96 services per conception.

Louca and Legates (1968), pointed out that yield per unit time, milk per day or year, was more important economically than was total lactation yield. With shorter intervals, milk production per day of life was definitely higher (Britt, 1975). Averge daily milk production from beginning of current lactation to day 150 of subsequent lactation (including the dry period) was the same for both early and late bred groups (Schneider et. al.,1981).

6. Herd Size and Housing

As the number of cows per farm continues to increase, there has been more emphasis placed on the possible effect of size of herd on pregnancy rate after first service and other methods of measuring herd fertility. It has been concluded by almost all investigators that the pregnancy rate decreased with the increased size of the herd (De Kruif, 1978).

The housing system has been shown to affect pregnancy rate after the first insemination, with the average rate being higher when systems were adopted in which the cows had freedom of movement, than it was in those systems in which the cows were restrained in stalls. This difference was found to disappear during the grazing season (De Kruif, 1978). Kiddy (1977) found that freedom of movement increased the intensity of the signs of estrus. De Kruif (1978) reported on papers by Kordts and Gravert (1972) and an earlier one by himself De Kruif (1977), that freedom of movement also stimulated the onset of ovarian activity after parturition, particularly in primiparous animals.

Housing is also important at time of parturition, and can have a great effect on subsequent fertility. Parturition and the initiation of high milk secretion places great stress on the animal and at this time the dairy cow is most susceptible to infection, particularly retained placenta and endometritis (De Kruif, 1978). De Kruif (1978) reported that retained placenta and endometritis would result in 5 - 10% lower pregnancy rates after the first insemination and a larger interval between parturition and conception.

7. Climate and Season

High temperature and humidity results in less marked signs of estrus and reduced conception rates, as well as increased rates of embryonic death. Wiersma & Stott (1969) suggested that increased progesterone secreted from the adrenal in response to a stressor, caused an imbalance of hormones such that the uterus and embryo became incompatible. Ulberg & Burfening (1967) studied the effects of temperature on fertility and found that uterine temperature on day of insemination was inversely related to fertility. They found that pregnancy rates declined from 61% to 45% as rectal temperature at 12 hours post-insemination increased 1°C.

In a study of the effects of early and late breeding Schneider et al. (1981), animals were divided into two groups by calving season. Animals calving during the pasture season, May through October were compared to those calving from November through April. No difference was noted between these groups in onset of estrous. However, animals in the late bred group required more services per conception and had longer calving intervals than cows calving in the non-pasture season. Cows calving in the pasture season produced more 4% fat-corrected milk than cows calving in the non-pasture season and were still losing weight between 60 and 80 days post-partum.

Other climatological factors affecting reproduction include: solar radiation, atmospheric pressure, precipitation and day length. Gwazdauskas et al., (1975) ranked the five most important climatological factors as follows:

1. Maximum temperature day after insemination,
2. Rainfall day of insemination,
3. Maximum temperature day of insemination,
4. Solar radiation day of insemination
5. Minimum temperature day after insemination.

The results of insemination vary from month to month and in temperate regions pregnancy rates are highest in the spring and decidedly less satisfactory during the autumn and winter months (De Kruif, 1978). This seasonal relationship to conception was noted by many other authors as well (Rosenburg et al., 1977; Stott, 1961; Gwazdauskas et al., 1975; Marion and Gier, 1968; Thatcher, 1974; Thatcher and Roman-Ponce, 1980).

8. Choice of Bull

The sire chosen to mate with a particular female in the herd becomes important in a number of ways. First of all, ease of calving must be taken into consideration. Recently, there has been much interest in this factor in the ratings of various sires at many A.I. Units. If the bull chosen to mate with heifers or small cows sires large calves, this may lead to dystocia, which in turn may lead to death or severe injury to the dam, retained placenta, endometritis and adhesions, which may be detrimental to subsequent fertility (Hansen, 1966; Rasbech, 1967; Williams, 1968; and Foley et al., 1972).

Secondly, fertility decreases with increased degree of inbreeding. However, this may be overcome in future generations by an

eventual outcross and subsequent increased hybrid vigor (Foley et al., 1972).

Finally, as mentioned earlier, there is variability in the fertilization capabilities of various sires.

9. Pathological Causes of Infertility

The pathological causes of reduced reproductive performance can be divided into three groups: those that affect only the reproductive organs; those that affect the reproductive tract and other organs; and those that decrease the general health of the animal and thereby adversely affect reproduction.

These would include the veneral disease such as those caused by Vibrio fetus, Trichomonas fetus and Campylobacter fetus, brucellosis caused by the bacterium Brucella abortus and leptospirosis caused by members of the genus Leptospira. Other infections such as IBR-IPV and BVD-MD may lead to reduced fertility. In most surveys at least 70% of bovine abortions are of unknown cause (Huck and Lamont, 1979).

Also included in individual pathological conditions are such conditions as anestrus and cystic ovarian disease. Anestrus, because of its high incidence and resulting losses, is a most costly infertility problem. Zemjanis (1961) classified anestrus as follows:

1. Pre-service Anestrus which included all animals that had not been observed in heat at the time of planned breeding and included post-partum cows as well as heifers.

2. Post-service Anestrus which represented animals that had failed to conceive and yet were not observed in heat.

Zemjanis (1980) found that in herds with averge fertility there was a 12.6% incidence of pre-service anestrus and 30.8% incidence of post-service anestrus.

The economic impact of anestrus becomes obvious when one considers that the minimum time lost because of post-service anestrus could be arbitrarily set at 42 days, corresponding to two estrous cycles. Pregnancy diagnosis at 45 days would increase this to three cycle lengths or 63 days. Zemjanis (1980) assumed that a minimum of 30 days was lost for each case of pre-service anestrus.

Ovarian cysts are a common clinically recognized endocrine cause of infertility in dairy cows. Surveys indicate that 5% - 10% of dairy cows are affected during the post-partum and breeding periods (Sequin, 1980). Zemjanis et al., (1961) made over 20,000 genital examinations from 1955 to 1960 and found abnormalities involving the ovaries represented the majority of detected genital defects (54.3%) and were found in 7.9% of all examinations. Cystic degeneration of the ovaries was the most prevalent abnormality diagnosed. Follicular cysts were observed in 4.0% and luteal cysts in 1.7% of all examinations (Zemjanis et al., 1961).

10. Nutrition and Reproduction in Dairy Cattle

Nutritional deficiencies and imbalances are frequently implicated as the cause of infertility in cattle. (Oxenreider and Wagner, 1971;

Foley at al., 1972; Morrow, 1980a, 1980b; Butler et al., 1981). A ration has its greatest effect on fertility before puberty, before and after breeding, and before and after parturition (Morrow, 1980b). When a ration is affecting reproduction, it is frequently the result of a deficiency of more than one nutrient, making it difficult to evaluate the effects of a specific nutrient on fertility (Morrow, 1980b). Observable signs of deficiency are variable, depending upon the degree of deficiency. Energy in moderate deficiency reduces fertility, while a severe deficiency results in anestrus (Morrow, 1980b). Generally, the first symptom to appear when a diet is inadequate is a decrease in milk production, with fertility being affected at a later stage (Morrow, 1980a).

The nutrients required for reproduction are those required for many other bodily functions and it is doubtful whether there is any single nutrient required for reproduction that is not also required for growth and milk production (Hafs and Boyd, 1973).

Deficiencies and excesses of a large number of feeds, minerals and trace elements and vitamins have been discussed in the literature as affecting reproduction and opinions on their effect differ markedly (Foley et al., 1972; Morrow, 1980 a & b). Different localities and soils produce specific local deficiencies and excesses (Morrow, 1980a).

De Kruif (1978) reported on papers by Adler and Trainin, (1960), and Lotthammmer and Ahlswede, (1973), who suggest infertility may be associated with the consumption of phytoestrogens from plants such as, clover, alfalfa and sugar beets.

High milk yield and its effect on the energy balance in the early post-partum dairy cow has also been shown to decrease fertility (Butler et al., 1981). Ruder et al. (1981) in a comparison of infection rates for protein adequate and protein deficient cows, found an infection rate of 52.2% and 48.1% respectively at 25 days post-partum.

11. Ideals and Requirements

It is apparent now, that fertility of a cattle population may be affected by an exceptionally large number of factors which often interact so that a correct interpretation of each individual factor is usually impossible.

The various investigations on days to first estrus, days to conception, calving interval, non-returns, services per conception and interval from first service to conception show that these measurements of additive genetic differences in fertility among cows are small. Thus selecting for improved breeding efficiency by these measures has little to offer dairy cattle breeders.

De Kruif (1978) listed the following as "ideal" values to strive for:

- a pregnancy rate of 80% after the first insemination
- an average of 1.3 inseminations per conception
- an average interval of 85 days between parturition and conception.

In actual practice it will be next to impossible to obtain these values as one or several factors will invariably exert an adverse effect

(De Kruif, 1978). It is understandable that reproduction does not reach an optimal level on a large number of cattle farms, most often due to inadequacies in management.

Melrose (1979) concluded that improved reproductive efficiency depends on being able to breed at the correct time, preferably on a pre-determined date, to know they are pregnant and that they will give birth at a stated time and detect a lowered or lowering of fertility to allow culling or treatment.

Therefore, the producer requires:

A. a reliable means of detecting estrus

B. a proven system for estrus and/or ovulation control

C. a pregnancy diagnostic technique readily applicable under field conditions and at a date as early as possible post-breeding to allow rebreeding

D. a fertility control program including access to clinical expertise to allow diagnosis of fertility problems (Melrose, 1979).

12. Pregnancy Diagnosis

The main use of pregnancy diagnosis in the cow is to enable the non-pregnant animal to be recognized as early as possible so that the time lost as the result of infertility may be reduced by the application of suitable treatment (Heap et al. (1976)). Norman (1982) listed five benefits of early accurate assessment of bovine reproductive rates:

1. Detection of estrus can be simplified by focussing the manager's attention on those cows likely to return to heat;

2. Breeding plans can be used more efficiently;
3. Non-producing cows can be culled early;
4. Fertility testing of bulls is simplified; and
5. Diagnosis of reproductive problems is facilitated.

Methods employed such as, observing an animal for a return to service and confirmation of pregnancy through clinical diagnosis based on rectal palpation, both lead to the possibility of days lost and the corresponding increase in cost of production on the basis that:

1. Animals which are not pregnant but show no estrus lead the farmer to the erroneous conclusion that they are pregnant. Hoffman et al. (1976) found this to be the case in 15.6% of the total animal population in his experiment.
2. Animals which are pregnant but show signs of estrus lead the farmer to the erroneous conclusion that they are not pregnant, and, if care is not taken during the second insemination, the insemination rod passing through the cervix may cause the abortion of the earlier pregnancy. During a 21 day period of constant observation seven per cent of pregnant cows exhibited standing estrus (Williamson et al., 1972).
3. The clinical diagnosis cannot be performed at an early enough time post-breeding.

Although there have been claims that by rectal palpation at 8 to 19 days after service, and again at 23 to 30 days, it was possible to diagnose pregnancy or non-pregnancy with 85% accuracy (Ludwick and Rader, 1968), in practice, the clinical examination is often delayed

until after the definitive allantochorionic placenta has formed at about day 45 post-coitum (Heap et al., 1976). For most dairymen it is not practical to have a veterinarian come out each time a cow reaches day 45 post-breeding. Most frequently they would be requested on a monthly basis and consequently the average rectal examination would occur at 60 days post-coitum. Rectal palpation for pregnancy has several limitations, some of which are:

1. It requires much practice to develop and maintain a reliable expertise for palpation and provides invalid results before first estrus;
2. It requires a fair amount of restraint and thus stresses the animal; and,
3. It is an invasive procedure that can result in rectal perforations, ovarian adhesions, ruptured corpora lutea, and abortions (Studer, 1969 and Norman, 1982).

A survey on the use of veterinary pregnancy diagnosis in 767 herds in England and Wales in 1969 (Milk Marketing Board, 1969) and of 1692 dairy farms in 1979 (Newton et al., 1982) reveals some interesting trends. The population of herd owners using veterinary pregnancy diagnosis on more than half the herd increased from 9.8 per cent to 14.2 per cent, and the proportion using them on some of the herd increased from 22.8 per cent to 43.8 per cent. The proportion not using the service at all had decreased from 67.4 per cent to 42 per cent by 1979, indicating a greater awareness on the part of the dairy farmer for increased reproductive efficiency.

B. Milk Progesterone For Monitoring Fertility

1. Plasma Progesterone Concentration

Plasma progesterone measurements have been made for a number of years (Stabenfeldt et al., 1961). A striking difference in peripheral plasma progesterone levels between pregnant and non-pregnant cows was observed 19 days after insemination (Shemesh et al., 1968).

In the cow it is essential that the secretory function of the corpus luteum be maintained for the greater part of gestation, since the placenta produces an insufficient amount of progesterone to maintain pregnancy in the absence of the ovary (Heap et al., 1976). During gestation, the concentration of plasma progesterone is similar to that in the mid-luteal phase of the estrous cycle (Stabenfeldt et al., 1970) and it has been used to confirm pregnancy on about day 22 after mating in cows that have not returned to estrus within 21 days of insemination (Robertson and Sarda, 1971). In the non-pregnant animal, the corpus luteum regresses on about day 17 or 18, while in pregnancy, the luteolytic property of the uterus is neutralized, and the corpus luteum survives (Heap et al., 1976).

2. Milk Progesterone Concentration

In the early 1970's, it was established that progesterone could be found in milk and that its concentration there reflected the variations of the estrous cycle and the plateau of pregnancy (Laing and Heap, 1971; Heap et al., 1973; Heap et al., 1976).

Evidence of this hormone was intitially obtained by combined gas chromatography - mass spectrometry, a technique which provided definitve

identification of a compound behaving like progesterone in cows milk during pregnancy (Darling et al., 1972). Laing and Heap (1971) first suggested that progesterone concentration in milk might provide a means of early pregnancy diagnosis in cattle.

In 1973, Heap et al., reported on a simple and rapid radioimmunoassay (RIA) which would measure directly the progesterone concentation in milk. They used the generic term, "PROGESTAGEN", in preference to the specific name of the parent steroid, progesterone, since their study showed that the concentration measurements also included an unidentified compound(s), which were not readily separable from progesterone, and were probably closely related to it.

Since the availability of RIA, milk progesterone levels have become one of the major hormonal parameters used to monitor reproductive efficiency, stimulating extensive laboratory investigations and elaborate field trials (Heap et al., 1973; Heap et al., 1976, Booth et al., 1976; Bishop et al., 1976; Hoffmann et al., 1976, Pennington et al., 1976; Shemesh et al., 1978, Foote et al., 1979).

3. Milk Progesterone and Pregnancy Diagnosis

Milk progesterone levels reflect ovarian luteal function, not the presence of a conceptus (Norman, 1982). Heap et al. (1973) felt that a limitation of the diagnosis of pregnancy from plasma progesterone concentration lay in the fact the progesterone values in the mid-luteal phase of the normal cycle in the cow resemble those of gestation. They therefore sampled, using sequential testing of milk progesterone

concentration for diagnosis, at a time when the difference between pregnant and non-pregnant cows is probably at its greatest (20, 24 and 28 days) and, which also allows for the individual variations in the length of the normal 21 day estrous cycle. A further sample on day 60, or about the time of routine clinical diagnosis, was also examined.

Embryonic mortality, the incidence of which ranges from 6.4% - 12% (Bulman and Lamming, 1979, Foote, et al., 1979; Holness et al., 1981., Ball, 1982), would be expected to lower the numbers of animals diagnosed pregnant at 60 days, compared to the number diagnosed pregnant at an earlier date.

The results of Heap et al (1973) given below, show a highly significant difference in milk concentration of pregnant and non-pregnant cows, suggesting that this test may form the basis of a method of pregnancy diagnosis. Milk samples were taken at the afternoon milking and represent a pooling of the first milk from all four quarters.

4. Milk Progesterone Pregnancy Diagnosis—Commercial Operation

Following publication of the results of Heap et al. (1973), Wickham Laboratories Limited, an organization in the United Kingdom already providing pregnancy diagnostic services in other classes of livestock, set up feasibility studies on routine pregnancy diagnosis. The results of these trials (Bishop et al., 1976) showed they could predict non-pregnancy with greater than 95% success. Prediction of pregnancy was more complex and their success rate was correspondingly lower. Nevertheless, as the non-pregnant cow is more a matter of

Table 2

Milk progesterone concentrations (ng/ml) in pregnant and non-pregnant cows measured by rapid radioimmunoasay

(Method B; antiserum 5-49 No. 6).
No. of observations in parenthesis.

Days After Estrus of Fertile Mating	Non-Pregnant	Pregnant	P Value
20	3.4 ± 0.69 (29)	7.0 ± 0.76 (37)	< 0.01
24	3.9 ± 1.33 (22)	7.4 ± 0.85 (36)	< 0.05
28	2.8 ± 0.74 (19)	7.6 ± 0.85 (37)	< 0.001
60	1.5 (2)	8.7 ± 0.96 (37)	-

from Heap et al., 1973.

concern than the pregnant one, the imbalance is in the right direction. Consequently, a commercial service was launched in June 1974 (Bishop et al., 1976).

Sample bottles and preservative were provided to prospective clients, with the request that the whole milk sample be taken on day 21-23 after insemination. Problems encountered included variable sampling times from ten days onward, samples of foremilk only, samples bearing little relation to the cow specified (in some milking systems), samples inadequately mixed with preservative, and over exposure of some samples to sun or heat sources. Submission forms were redesigned to include sample data and service date, to alleviate some of the problems, and to impress upon the user the fixed relationship between the two.

In their conclusion, Bishop et al. (1976) felt that the early diagnosis of non-pregnancy was invaluable, especially as a screen in larger herds. They also felt that better awareness of the service would improve its commercial success.

The Milk Marketing Board (UK) launched a commercial pregnancy testing service on October 1, 1975. They began by recommending sampling on day 28, and later brought this forward to 24 days post-insemination. They also wished to standardize the sampling procedure, realizing that variations in the butter fat content would result in variations in the progesterone content. Therefore, they asked that samples be taken at the afternoon milking and be of whole milk (Booth et al., 1976).

After six months, there were 1200 members utilizing the service, and they found that the service was of particular appeal to the owner of larger herds, with 88% of the users having more than the United Kingdom average of 40 cows, and 9% having more than 200 cows (Booth et al., 1976).

Booth and Holdsworth (1976) reported they were receiving samples at the rate of approximately 7000 a month, and that 85% of the results were dispatched to the farmers within two days of receipt, five per cent of samples were re-assayed for various reasons and the results of these, together with the ten per cent completed but on the same results cards, were returned within four working days of receipt.

In the report they stated that the majority of the farmers used the service primarily for the detection of non-pregnant cows.

Booth et al. (1976) also reported on field trials they conducted. They noted in the four herds they were monitoring that there appeared to be somewhat different distribution characteristics of the progesterone levels of the cows. These were as follows:

Table 3
Mean progesterone level of positive cows from four East Anglia Herds sampled 24 days post-breeding

Herd	No. of Cows	Mean Progesterone ng/ml (± S.E.)
A	24	12.7 ± 2.6
B	15	13.0 ± 2.1
C	20	22.8 ± 2.0
D	10	19.3 ± 2.6

Booth et al. (1976).

In a further attempt to determine typical levels, they randomly selected six herds having more than 40 test results, and analysed the progesterone levels of the positive cows.

Table 4
Mean progesterone level of positive cows (March 1976) selected at random sampled 24 days post-breeding

Herd	No. of Cows	Mean Progesterone ng/ml (± S.E.)
1	60	23.3 ± 1.3
2	51	21.6 ± 1.3
3	34	29.0 ± 2.6
4	58	27.8 ± 1.7
5	43	23.9 ± 1.4
6	54	27.2 ± 1.5
Total	300	25.3 ± 0.7

Booth et al. (1976).

They noted Herd No. 3 had a high proportion of both negative (18.8%) and doubtful (10.4%) test results and subsequently discovered that this owner was using one of the new drugs for estrus synchronization.

In an attempt to determine what might have caused the extremely low levels of herds A & B of the first set of trials (Table 3), they reexamined the four herds again.

They found that since herd B was milked on a pipeline system, it was therefore impossible to obtain representative samples of whole milk without the use of cow meters, and a large number of samples submitted had been of foremilk. They could find no explanation for the low levels in herd A.

On the basis of field trials and, with the equipment and procedures they used, Booth and Holdworth (1976) found there was a distinct demarcation between positive and negative cows at the level of 6.5 ng progesterone/ml milk. They allowed a doubtful area of 1 ng/ml on either side of this level and repeat tested all doubtful cows (1.2%) at day 42.

An interesting aspect brought out in this report, was the initial apprehension in the veterinary profession on the announcement of the new milk test for pregnancy. It was later determined that there was an increase in requests for assistance in treating infertility cases at an early stage when it was still economical to do so. Other veterinarians were found to be using the test as a regular surveillance, which they later followed with rectal palpation (of particular value to herd where early embryonic/fetal death is a problem). They reported that it is

also recommended by veterinary surgeons where poor estrus detection is suspected for which additional samples would be taken on day of insemination. And finally, they noted that veterinarians have also used the test for goats as they are too small for rectal examination.

In concluding their paper Booth and Holdsworth (1976), felt that the widespread application of the test to the national herd for the early detection of pregnancy would play a major part in the reduction of losses due to infertility and in increasing the profitability of dairy farming.

5. Further Experiments Using Milk Progesterone Pregnancy Diagnosis

Heap et al. (1976) continued experiments on the use of milk progesterone concentration as a pregnancy diagnosis. They found that the concentrations were greater in full milk taken at the afternoon milking than in first milk taken at the morning milking. A correlation of the concentration of progesterone in milk with fat content in samples with low progesterone was found but not in those with high progesterone values. The success rate of the pregnancy test from a single milk sample at 21, 24, 28 or 42 days after insemination ranged from 77.5% to 85.8% for pregnant cows and 85.7% to 100% for non-pregnant cows. Combining the results of two field trials, the highest success rate was found at 24 days when 142 of 176 pregnant cows (80%) and 25 of 25 non-pregnant (100%) were correctly diagnosed from either samples of first milk from any healthy quarter at morning milking (Field Trial 1), or samples of whole milk collected at mid-day or afternoon milking (Field Trial 2).

Measurements of known amounts of progesterone (0, 5, 10, 20 and 40 ng/ml) added to milk samples with a low endogenous concentration (0 to 4.3 ng/ml), showed that there was a tendency to overestimate low values and underestimate high values (Heap et al., 1976) as previously found with a competitive protein binding procedure (Heap et al., 1973).

The milk test of pregnancy has been developed to use relative rather than absolute concentrations of progesterone for the correct identification of pregnant and non-pregnant animals (Heap et al., 1976). The fact that progesterone and milk fat concentrations showed a highly significant positive correlation ($r = 0.97$) (Hoffmann and Hamburger, 1973) in cows with an active corpus luteum, led Hoffmann et al. (1976), to the decision to use only the fat-rich strippings, or, if this was not possible, the whole milk for assay purposes.

Of 133 animals classified, Hoffmann et al. (1976), found the general agreement in the group of animals diagnosed pregnant was 77%, while those diagnosed as non-pregnant with values less than 2 ng/ml had 100% agreement.

They concluded that the only information obtained which can substitute completely for clinical examination is the exclusion of pregnancy when progesterone is below 2 ng/ml milk (Hoffmann et al., 1976).

In order to decrease the range of the questionable classification, Hoffmann et al. (1976), developed a method of measuring the progesterone in milk fat. Changes in progesterone production during the cycle were well reflected with the range in values falling between 0.15 ng/10 µl milk-fat at estrus and 2.5 ng/10 µl milk fat during the

luteal phase (Hoffmann et al., 1976).

Eastman (1979), also felt that the accuracy of the progesterone test could be improved by using a progesterone-in-milk-fat value. He used 1.0 ng progesterone/10 µl milk-fat as his discriminatory level and verified negative diagnosis (less than 1.00 ng progesterone/10 µl milk-fat) by a return to service, and positive diagnosis (greater than 1.00 ng progesterone/10 µl milk-fat) on a 60 day non-return basis. His results do not show much improvement in accuracy however, with positive diagnosis having only 83.3% agreement on 25 of 30 cows, and the negative diagnosis being in 100% agreement in 24 cows, with samples collected on day 21 after breeding.

Pennington et al. (1976), found the use of extracted or non-extracted milk for progesterone analysis gave similar results for pregnancy diagnosis, and that diagnosis by concentration of progesterone in milk was not improved by expressing progesterone as progesterone per unit fat, or progesterone per milking. Eliminating the extraction step reduced labour required for the RIA. They examined 508 cows and found that diagnosis of cows as pregnant and non-pregnant by milk progesterone on day 21 post breeding had 76% and 98% agreement, respectively, with diagnosis by palpation or return to estrus as verification of results.

Examining the best time to sample for pregnancy post-breeding, Pennington et al. (1976), found pregnancy diagnosis by progesterone determination in milk on day 21 or day 23 post-breeding had a greater agreement with diagnosis by palpation than diagnosis by milk progesterone on day 19, 25 or 27 post-breeding.

Pennington et al. (1976) compared results from the 5 major dairy breeds and determined that breed of cow did not affect the accuracy of diagnosis.

Zaoral et al. (1982) studied the effects of sampling time on the accuracy of the milk progesterone pregnancy test. They used three sampling variations, confirming pregnancy with a rectal examination 60-90 days post-insemination:

1. Foremilk and whole-milk sample on day 23 for 1691 cows, resulting in 87.7% correctly diagnosed non-pregnant, 67.3% correctly diagnosed pregnant, for an overall agreement of 73.3%.
2. Sampled on day of insemination or day 1, day 19 and day 23.

No significant difference in the proportion of the results in agreement in both tests (progesterone test (PT) vs. clinical examination) were found in the group of non-pregnant cows between sampling variation (1) and variation (2) or the different combinations of milk sampling in variation (2). The highest per cent agreement of results (91.9%) was obtained by a single sample on day 23. In the pregnant group, the highest per cent agreement (78.7%) was obtained in the group sampled all three times. This was significantly higher than any other combination. The results of the group of pregnant cows influenced the overall results, the range being from 62.9% for one sample on day 19 to 81.2% for all three samples.

3. In variation (3) three samples were again obtained. This time however, there were only two sampling days per week (Monday and Thursday).

The first sample was taken on day 19 to 22, the second on the following sampling day (day 22 to 25) and the remaining sample the following week (day 29-32).

The differences between all the combinations in the group of non-pregnant cows were insignificant, while in the groups of pregnant cows, the single sample showed significantly poorer agreement than all combinations of two samples and in comparison with the three sample combination. The range in agreement was from 70.6% to 83.9%.

The significance of the overall results was similar. An increased number of samplings increased the proportion of correctly PT-determined non-pregnant cows. Hence, the proportion of incorrectly PT-determined cows was reduced in the group of pregnant cows (Zaoral et al., 1982).

These results are contrary to those reported earlier by Heap et al. (1976) and showed that the combination of two samples was more advantageous. Because variation (2) and variation (3) results were comparable, sampling could be restricted to two days a week reducing the laborious daily sampling and eliminating errors caused by missed samples.

Shemesh et al. (1978) found similar agreement using milk progesterone to determine pregnancy with 78% accuracy in predicting pregnancy and 100% accuracy in predicting non-pregnancy with an overall accuracy of 88% using afternoon foremilk samples taken on day 24. They used a highly specific-antisera providing minimal cross-reaction with other metabolites.

The average progesterone concentration was 5.1 ng/ml milk and ranged from 1.3 ng/ml to 15.9 ng/ml milk in lactating non-pregnant

cows and was 19.7 ng/ml milk and ranged from 7.1 ng/ml to 35.6 ng/ml milk in pregnant cows.

The results of this study support the opinion of those investigators who contend that the principle value of milk progesterone testing for pregnancy is to detect those cows which are not pregnant (Shemesh et al., 1978). The results of positive pregnancy assays from several countries with varying conditions of management, nutrition and level of production, indicate that with present techniques, positive assays cannot be expected to give an accuracy of more than 80%. In practical terms this means that under present conditions what is needed are more efficient methods for estrus detection and milk progesterone assay will have its chief application for early detection of non-pregnant cows, as well as an aid to clinical diagnosis of such conditions as cystic ovaries, subestrus, anestrus, and for monitoring changes in progesterone levels before and after gynecological treatment (Shemesh et al., 1978).

Holdsworth et al. (1979) discussed the problems entailed in a large scale application of the radioimmunoassay for progesterone. They found that the addition of the label immediately after the antiserum, abolishing the twenty minute incubation period, did not appreciably alter the results, the correlation between assays with and without incubation being highly significant ($n = 0.93$). They then tested the effects of aging the antiserum/label mixture from one to three hours, and found that levels fell throughout the period of the experiment, but the fall was not significant. Use of the premix improved the rate of

sample through-put significantly, and removed the necessity for one timed stage.

Holdsworth et al. (1979) attempted to identify sources of variation, both inter-assay and intra-assay, and concluded that the variation originates largely from the samples, and the observations of Van der Wiel et al. (1978) indicate that non-specific binding (NSB) could be implicated. They found NSB varies from sample to sample (ranging from 13.9% to 25.6%, mean 21.4%), but were unable to produce any reduction in assay variation by correction for NSB, or demonstrate any alteration in NSB by sample treatment (Holdsworth et al., 1979).

They concluded that the importance of assay variability is largely related to the application of the assay. If as in a pregnancy test, its main function is to distinguish high and low levels, then greater variation can be accepted than if absolute levels are being compared.

Stevens et al. (1981) found that an increase in temperature, and dilution of the milk sample both independently and, in combination, reduced NSB. The RIA of Heap et al. (1973) was modified to account for both increase in temperature and dilution. Stevens et al. (1981) also determined that this refinement in technique also gave better repeatability.

Booth et al. (1979), reporting on the use of the progesterone test for a pregnancy determination service offered in the United Kingdom since 1975, found that after three years of operation, more than 100,000 cows per year were being tested and 5.6% of farmers in England and Wales were using the service. A sample of large herds using the service found average accuracy rates of 84.5% and 97.0% respectively for positive and

negative tests. They found that the highly significant correlation ($r = + 0.84$) between proportion of positive tests in a herd and the accuracy rate of these tests, along with an analysis of insemination interval data, were good indicators of herds requiring veterinary investigation (Booth et al. 1979).

In their examination of milk progesterone as a diagnostic aid, Foote et al. (1979) found that under field conditions it was preferable to use last milk samples, as they tended to be the least contaminated, were highest in progesterone content and, could be taken after some lapse of time, if it was soon discovered that a sample was missed. On a total of 315 cows they reported a 98% accuracy for non-pregnant and 80% accuracy for pregnant with an overall accuracy of 87% (Foote et al., 1979).

Laing et al. (1979) sampled Friesian cows on days 38 and 46 after service, the sample day chosen so as to cover the known normal variation in cycle length, from 17 to 25 days, so that any cow returning to estrus after two cycles should be detected and, because the sample times were later than the end of the main period of embryonic mortality. They found the accuracy for positive diagnosis from milk samples to be increased to 95.2% indicating the inaccuracies caused by embryonic mortality had been to some extent overcome (Laing et al., 1979).

Recognizing that the error factor of greater than 20% in positive pregnancy diagnosis based on milk progesterone may be due to either luteal phase progesterone, or early embryonic abortion (Booth and Holdsworth, 1976; Heap et al., 1976; Hoffmann et al., 1976; Pennington et al., 1976), Shemesh et al. (1981) used progestin impregnated vaginal

sponges (PIVS) in order to diminish the wide spread range in the time of return to estrus usually seen in inseminated cows which are not pregnant (Booth and Holdsworth, 1976; Heap et al. 1976; Hoffmann et al. 1976; Pennington et al., 1976).

PIVS were given six to seven days following insemination and removed on day 17 following insemination to 50 Israeli-Friesian cattle. Ninety-six animals acted as controls. Milk samples were obtained on day 21-24 after estrus and assayed for progesterone using fat-free milk while pregnancy was confirmed at six to seven weeks by rectal palpation. In contrast to the control group in which 11 of 52 (21%) cows were incorrectly diagnosed as pregnant, 100% of 30 cows diagnosed pregnant with PIVS inserted were correctly diagnosed. The accuracy of the test for negative pregnancy was 100%, regardless of treatment (Shemesh et al. 1981).

An interesting aspect of this experiment was discovered when the fertility rates of the two groups were examined. The fertility rate for the control group was 43% and the PIVS group was 60%. Though the sample is too small to draw any firm conclusions, this indicates a possible beneficial effect on fertility (Shemesh et al., 1981).

The majority of the previously described reports have employed a specified concentration of progesterone in milk, or milk fat, to discriminate between only two categories, pregnant and non-pregnant cows, and have used clinical diagnosis, by rectal palpation, as the reference standard, to determine accuracy. Gowan et al. (1982) found similar results, namely 76.9% of positive and 93.8% of negative diagnosis, confirmed by rectal palpation, or a return to estrus 28 - 150

days after insemination, when 3014 cows in 394 herds were sampled 23 days post-insemination. When those animals whose samples on day of estrus showed a greater than normal concentration of progesterone were excluded, the accuracy of the pregnant diagnosis increased by 7.1% to 84% and the non-pregnant diagnosis increased by 3.1% to 96.9%.

In this experiment dairymen submitting samples were required to record the following information: date of insemination, diagnosis by the herd veterinarian after palpation of the uterus, and date of uterine examination, or a record of the date of return to estrus, if estrus was observed after the sample was taken on day 23. Milk samples were collected on day of insemination, and day 23 after insemination, and in cases where the cows returned to estrus prior to day 23, the samples were not analysed.

From this information Gowan et al. (1982) showed an increase in agreement between the two methods of diagnosing pregnancy as time between insemination and palpation of the uterus increased, observed that the accuracy of diagnosis by palpation of the uterus increases with time during the early stages of gestation, and concluded that from their data, the most accurate method currently available to diagnose pregnancy before 50 days of gestation is concentration of progesterone in milk collected 23 days post-insemination.

Their data also showed that as the concentration of progesterone on day 23 increased, the probability that pregnancy would be confirmed by palpation increased until the maximum agreement between the two

methods of diagnosing pregnancy was 85% and concentration of progesterone was 9.51 to 10.0 ng/ml.

Assigning a probability that pregnancy will be verified after 50 days or more of gestation by uterine palpation along with the absolute concentration allows the dairyman to decide how much effort should be expended on each individual animal for detection of estrus and emphasizes a realistic expectation of the success of a pregnancy diagnostic service (Gowan et al., 1982).

6. Estrus Detection Using Milk Progesterone Assay

Plasma progesterone concentration returns to a basal level at parturition and remains at this level until the ovaries begin their cyclical function. Milk progesterone concentration also is at basal levels from parturition until ovarian activity resumes.

Researchers in 1979 studied 50 profiles and concluded a progesterone value of more than 5 ng/ml from hand-stripped aftermilk was indicative of luteal activity (vandeWiel et al., 1979). This was further refined by Ball (1982) who studying more than 3000 milk progesterone profiles used the parameter of at least two consecutive values $\geq$ 3.0 ng/ml as the first progesterone rise indicating luteal activity.

Resumption of luteal activity should occur within 30 days post-partum (vandeWiel et al., 1979). They found 12% of the animals they examined took longer than 30 days. Bulman and Wood (1980) in an examination of profiles of 533 dairy cows found that 4.9% had not resumed cycling by 50 days post-partum.

Estrus and successful AI are concomitant with a nadir of progesterone level; high progesterone concentrations are incompatible with fertility during this time. Karg (1981) estimated that progesterone should rise 4-5 days after estrus, indicating that ovulation and, in consequence, corpus luteum formation has occurred, and suggested that false timing of AI may be even more frequent than indicated by the progesterone assay, due to the fact that the average duration of estrus is 15-18 hours and standing heat at 9-10 hour is much shorter.

Shemesh et al. (1978) reported results of Mylrea (1962) which found that natural service gave better conception rates than artificial insemination, and reports of Günzler et al., (1973) and Appleward and Cook (1976) that at least part of the explanation for this difference may be ascribed to inaccurate diagnosis of estrus as reflected by progesterone concentrations in blood and milk of cows presented for insemination.

Hoffmann et al. (1976) stated that the measurement of progesterone in milk is an excellent way of identifying those animals which show no behavioural estrus 20 days after insemination and are not pregnant, or those which are inseminated during the active corpus luteum phase and thus cannot conceive. They found that 22% to 27% of the samples sent in for routine analysis were calculated to fall into these categories.

In an experiment, 13% of 299 animals with no chance of conception were identified at the time of insemination through analysis of progesterone in milk (Hoffmann et al., 1976).

Shemesh et al. (1978) compared the assessment of estrus by progesterone concentration in milk on day of insemination and assessment of estrus by a veteran herdsman and an experienced inseminator. Accuracy of estrus diagnosis by herdsman, inseminator, and milk progesterone level were, 84%, 93%, and 96%, respectively (Shemesh et al. 1978).

Sixteen percent of the cows presented for insemination by the herdsman were not in estrus as judged by milk progesterone levels. In two other studies, between 10% and 20% (Appleward and Cooke, 1976), and between 14% and 26% (Hoffmann et al., 1976), were not in estrus when inseminated as judged by plasma or milk progesterone levels, respectively. Zaoral (1982) sampled cows on day of insemination and found 14% of the inseminations were performed outside of estrus.

Foote et al. (1979), in an experimental herd where the opportunity for heat detection was poor, found the pregnancy rate for first service was about 40%. An analysis of the milk progesterone records revealed that 98% of the cows were cycling normally, starting at or about 50 days post-partum. When inseminations were performed at a reported estrus which coincided with low progesterone values, the pregnancy rate was over 60%.

In a second experimental herd with a good programme of estrus detection and breeding, Foote et al. (1979) reported that in 19% of the cycles, estrus was reported when progesterone concentration in milk was high.

Using defatted milk samples, McCaughey & Cooper (1980) showed a negative correlation exists between levels of milk progesterone greater than 0.20 ng per ml defatted milk and calving rate. Eight of 96 cows in an experimental herd showed levels of progesterone much above the normal estrus level (more than 0.30 ng/ml) and failed to conceive. Of 1177 milk samples from cows being inseminated in commercial herds, 91 (7.7%) had levels of milk progesterone in excess of those observed at normal estrus.

The potential value of progesterone assay in monitoring for non-observed estrus or sub-estrus in the post-partum period needs further study, especially in relation to frequency of sampling, as this would seem to be the most beneficial area for its use (Melrose, 1979). Major difficulties arise in estrus diagnosis in up to 20% of cows and it has been suggested that up to 20% fail to conceive due to insemination at the wrong time, emphasizing again the possible use of hormone profiles to allow monitoring for such situations (Melrose, 1979).

Due to intensive husbandry along with increased herd size and reduced labour force, the requirement of time to reach 80% plus estrus detection would add to the existing unsocial working hours of the cowman. Melrose (1979) suggested a possible compromise in which we monitor in retrospect, by hormone assay, the efficiency of estrus detection, and when necessary, tighten up the routine. Where analysis shows poor estrus detection, there could be a place for aids such as heat mount detectors, to bring out estrus signs more clearly.

The benefits of recording symptoms rather than simply estrus was reported by Foote et al. (1979). They found, with improved management, only 5% of the cattle reported for insemination were in diestrus as compared to 19% previously (Foote et al., 1979).

Ball and Jackson (1979) used a milk progesterone determined estrus as the basis of a double insemination, in an attempt to reduce the calving interval of those cows which were cycling but not showing behavioural signs. Cows were observed for estrus six times per day between 5:30 a.m. and 10:00 p.m. and all signs were recorded at each observation period. Ka-Mar® heat detectors and Delta mate-markers® were also utilized. Milk samples were obtained thrice weekly and, progesterone levels in fat-free milk were determined. This information was used to predict the time of estrus at which insemination was due to within two to three days. Milk samples were taken daily beginning five days before the estrus was due and cows failing to exhibit estrus following a drop in progesterone to below 1.5 ng/ml fat-free milk were inseminated two and three days after the fall. Those showing signs were inseminated once at the appropriate time.

Of 55 cows inseminated on the basis of the fall in progesterone concentration, 33 or 60% conceived compared to 86 or 65.2% of the 132 inseminations on the basis of observed estrus. The difference in conception rate was not significant.

Of 3014 cows examined by Gowan et al. (1982) 352 or 11.7% had higher than normal progesterone concentration on day of insemination. However, an estimate of the frequency with which cows were bred during diestrus was derived from those cows for which the concentration of

progesterone was high and for which uterine palpation or a return to estrus indicated absence of fetus. They found only 3.5% of their survey met these qualifications and, therefore, represented the maximum frequency with which cows were bred during the estrous cycle when a functional corpus luteum was present.

7. Embryonic Mortality

Embryonic mortality, strictly interpreted, should refer to fertility losses during the embryonic period, i.e. the period extending from conception to completion of the stage of differentiation which, in the cow, occurs at approximately 45 days (Committee on Reproductive Nomenclature, 1972).

Using planned slaughter of repeat breeder cows, Ayalon (1981) determined that the critical period appeared to be soon after the embryo enters the uterus, six to seven days after service, when the morula is developing into the blastocyst. Ayalon (1973) has shown that day 7 rather than day 6 is the critical day on which embryonic death becomes evident.

Diskin and Sreenan (1980) investigated 246 beef heifers and determined early embryonic mortality occurs mainly between days 8 and 16 (Karg, 1981). Spontaenous embryonic losses afterwards (beyond the non-return day 21), seem to occur mainly between days 30 and 45 after AI, at the time when the attachment of the placenta to the uterus is becoming firmly established, Ball (1980).

Plasma and milk progesterone concentrations have been used to estimate embryonic loss. When progesterone evaluation on day of

insemination is included, accuracy of this approach is increased as you can eliminate those animals with a functional corpus luteum on day of estrus. However, this method does not overcome the problems associated with estrus during early pregnancy, estrus in cows with endometritis, nor will it detect the majority of embryonic losses, which are occurring before 15 days post-insemination (Ayalon 1981).

Ball (1982) examining over 3000 post-partum profiles for cows from 22 herds, determined detectable embryonic loss as those cows having progesterone levels rising within eight days of insemination and remaining high until at least 25 days after insemination, indicating that pregnancy had been established, followed by a subsequent fall in progesterone levels indicating that the embryo had been lost. This occurred in only 6.4% of the cows he examined.

Foote et al. (1979) assumed embryo/fetal mortality for any breeding interval exceeding 28 days up to the 75th day. In one experimental herd (herd A previously mentioned), the estimated loss between 28 days and 75 days was 22.7%, clearly overestimating the embryo mortality, as many cows in estrus before 28 days were missed according to normal cyclic patterns in milk progesterone. When they estimated embryo mortality on the basis of any milk progesterone cycle longer than 27 days after insemination followed by regular cyclical patterns the estimated loss was reduced to 7.2% (Foote et al., 1979).

Bulman and Lamming (1979) sampled 555 cows twice weekly and found that embryonic mortality occurred in 12% of the animals between 31 and 59 days after insemination. They compared the mean progesterone levels of 57 normal cows and 23 cows in which embryonic mortality occurred for

the first 30 days of pregnancy and found no difference. Following this, progesterone levels remained in the normal range in the latter group until a steep decline to basal levels indicated that embryonic mortality had occurred.

Holness et al. (1981) using plasma progesterone concentrations to study fertility, found 11% of 69 cows returned to estrus between 30 and 88 days after insemination. They also found no difference in mean plasma progesterone concentration from four to 46 days after insemination between cows that returned to estrus between days 30 and 88 after insemination and those that remained pregnant. Earlier, Holness et al. (1977) had found 10% of 90 cows suffered embryonic mortality between 26 and 81 days after insemination.

8. Milk Progesterone and Infertility

Lamming and Bulman (1976) collected twice weekly samples of whole milk from 300 cows in three commercial herds, from 14 days post-partum until they were diagnosed pregnant. Signs of estrus were recorded and AI was used. Results from the first 200 cows examined showed the interval from parturition to ovarian activity, as measured by a substantial rise in the milk progesterone levels, occurred prior to 20 days for 50% of the animals. Ninety-three per cent had shown ovarian activity by day 40, allowing at least one cycle prior to an optimal first insemination date of 65 days post-partum, and 15% produced milk progesterone profiles suggestive of subfertility.

Their data confirmed the problem of "silent" estrus. Only 30% showed estrus at the beginning of the first cycle, 63% after the first

cycle and 86% after the third. Excluding the first cycle, where overt estrus might not have been observed because of a lack of previous progesterone, only 77% of the remaining estrus periods were observed (Lamming and Bulman, 1976).

In a large proportion of cows, a small rise in milk progesterone lasting from six to nine days occurred preceding a full ovarian cycle. Anestrus was determined in seven per cent of animals showing persistently low levels of progesterone until after 50 days post-partum. Two per cent showed a pattern of persistent high milk progesterone for at least 30 days after a normal estrus and apparent ovulation, suggesting that luteolysis did not occur at the normal time of approximately 17 days after ovulation (Lamming and Bulman, 1976).

Bulman and Lamming (1979) sampled 37 cows once every 10 days beginning 25 days post-partum until the establishment of pregnancy and on the day of estrus. They determined that these provided sufficient data to allow an accurate assessment of herd fertility and management.

Of 125 ovulatory periods covered by sampling, estrus was observed in 91, giving an estrus detection rate of 73%. Nine cows had not been seen in estrus by 50 days post-partum, five of these were still acyclic and four were cycling but estrus had not been observed.

Parameters measured were the overall intervals from calving to first estrus (43.3 ± 4.51 days, n = 37), first service (78.7 ± 4.13 days, n = 37), conception (87.1 ± 4.51 days, n = 35) and the conception rate (1.26 ± 0.08 services per conception, n = 35).

The importance of abnormal patterns of ovarian activity on herd fertility was examined by analyzing twice weekly milk samples for

progesterone from 533 dairy cows from parturition until the re-establishment of pregnancy (Bulman and Wood, 1980).

Based on profile analysis, the animals were placed into the following categories:

a. normal - 77.5%*

b. delayed start to ovarian cycles - 4.9%

c. cessation of cycles - 5.1%

d. prolonged luteal activity - 1.9%

e. silent estrus - 10.7%

*The incidence of 'normal' cycles was 75% for first lactation, 80% for 2nd to 5th lactation and 65% for 5th to 11th lactation.

Animals in groups "b" to "e" were subdivided into treatment and control groups. The treatment for groups "b" and "c" were:

1. a single injection of 0.5 mg luteinizing hormone-releasing hormone (Hoechst) or,
2. progesterone-releasing intravaginal device (Abbott Lab. Ltd.) inserted for fourteen days.

For groups "d" and "e," treatment consisted of a single injection of 0.5 mg cloprostenol (Imperial Chemical Industries).

It was determined that none of the treatments reduced calving to conception interval, although the majority responded to treatment. Nearly half (47.8%) resumed cyclic ovarian activity within 20 days of calving and this increased to 92.4% by 40 days. Abnormal ovarian function of some sort was noted in 22.5% and had a highly significant interaction with age.

9. Milk Progesterone to Monitor Hormonal Treatment of Subfertility

In a study by Lamming and Bulman (1976), treatment was initiated in animals identified as anestrus by negligible progesterone levels up to 50 days post-partum and consisted of:

a. 0.5 mg Gn RH (Hoechst HOE 471) or

b. a progesterone releasing intravaginal device (PRID Abbotts).

Some animals were left untreated as controls to indicate the extent of spontaneous recovery.

Cows termed subfertile because of regular luteal activity as shown by progesterone but exhibiting no estrus in the follicular phase were treated with prostaglandin (PG) following two consecutive follicular periods without estrus, "silent estrus" (Lamming and Bulman, 1976).

Of seven animals treated with Gn RH, five responded with cyclic activity, although the response was variable, ovulated, and became pregnant. Generally, animals treated with Gn RH did not show immediate post-treatment estrus (Lamming and Bulman, 1976). Of seven animals treated with PRID, all responded with subsequent luteal activity, five showed post-treatment estrus and six became pregnant at the first or second post-treatment ovulation. A uniform rise in milk progesterone following removal of the device was noted, suggesting that a timed insemination might be used. Since post-treatment fertile estrus occurred after PRID insertion, Lamming and Bulman (1976) felt that there was a potential to establish an earlier pregnancy with this device

compared to Gn RH injection. Of nine animals treated for "silent" estrus with PG, eight showed post-treatment estrus and seven became pregnant after insemination.

Bulman and Lamming (1977) collected milk samples from over 300 dairy cows from parturition to confirmed pregnancy by milk progesterone test. A progesterone profile was plotted for each animal. If the progesterone in milk of unmated cows remained greater than 13 ng/ml for more than 30 days, the animal was classified as having an abnormally long period of luteal activity, and was assigned to either a control, or treatment group which received a single injection of prostaglandin.

Six animals or 1.5% of those surveyed were found to have prolonged luteal activity. Of the three animals treated, all responded to a single injection of prostaglandin with complete luteolysis. A further 7% of cows studied were seen in estrus between 30 and 70 days after insemination. It is possible some of these could have experienced prolonged luteal activity without being pregnant.

One important aspect of this paper is that Bulman and Lamming (1977) state that prolonged luteal activity can occur in the absence of any apparent clinical abnormality.

In another study, the anestrus syndrome was defined as no estrus occurrence by post-partum day 60 (post-partum anestrus), or by 21 to 24 days after insemination, if a negative early pregnancy test based on milk progesterone concentration was obtained (post-insemination anestrus) and two treatments, gonadatropin releasing hormone (Gn RH) and

prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analog were tested (Humblot and Thibier, 1980).

In the first of their experiments, Humblot and Thibier (1980) divided the post-partum anestrus cows (those not seen in estrus for 60 days post-partum) into four groups:

Group 1 - Control group consisted of 18 non-treated anestrus animals.

Group 2 - 131 animals with a functional corpus luteum shown by progesterone values greater than 2 ng/ml milk or 71.5 ng/ml plasma were treated with $PGF_{2\alpha}$ and inseminated systematically 72 to 96 hours after injection.

Group 3 - 21 animals with true anestrus with milk progesterone values less than 2 ng/ml or plasma value less than 1.5 ng/ml in two samples taken at 10 day intervals, were treated with one injection of Gn RH and inseminated at the observed estrus.

Group 4 - 14 animals with true anestrus were given one injection of Gn RH and according to ovulation occurrence (as shown by progesterone concentrations in blood or milk) were assigned to one of the following treatment regimens. Cows observed in estrus in the ten day period after treatment were inseminated. Cows not observed in estrus and with low progesterone values were treated again with Gn RH. And finally, cows not observed in estrus, although ovulation had occurred, were treated with $PGF_{2\alpha}$.

Humblot and Thibier (1980) found 29% of the animals showed post-partum anestrus:

Control or Group 1 had a mean interval between parturition to first service (1st estrus) of 101 ± 21 days with a conception rate on this service of 44%.

Group 2 were bred 27 days before the controls were bred, with a conception rate similar to that of controls. The mean interval between treatment and conception was 41 days.

Group 3 had a mean interval between parturition and first insemination of 101 days. The interval between first Gn RH injection and conception was 53 days.

Group 4 had a mean interval between first Gn RH injection and conception of 34 days.

On the whole, more cows in the treated groups were inseminated within 90 days (42% vs 16%) after parturition and conception occurred 17 to 25 days earlier than in the control anestrus cows. Of the 29% of the cows showing the anestrus syndrome 60 days after parturition only 21% did not show ovarian activity.

In the second experiment, Humblot and Thibier (1980) found 71 non-pregnant cows were anestrus 21 to 23 days after insemination by low milk progesterone and were not observed in estrus.

Group 1 - Control group of 28 untreated animals.

Group 2 - 43 Animals were injected with $PGF_{2\alpha}$, 10 to 15 days after the date of negative early pregnancy diagnosis and were inseminated at 72 and 96 hours post-treatment.

The treatment group conceived 24 days earlier than did the controls.

These authors felt that the savings due to the reduction in mean calving interval would more than cover the cost of the investigations and treatments.

SUMMARY

To summarize the preceding, it has been shown that the dairyman is faced with many problems and factors including anatomical, genetical, physiological, pathological and management factors to consider when trying to optimize fertility in the dairy herd.

Modern architectural engineering in agriculture can modify the environmental effects, feed analysis and ration formulation can eliminate the nutritional effects, and the many research reports on management strategy can provide the dairyman with reproductive goals to strive for.

Through the use of radioimmunoassay of progesterone in easily obtained milk samples the dairyman now has a means of early accurate assessment of bovine reproductive status, the benefits of which as stated by Norman (1982) are:

1. Detection of estrus can be simplified by focussing the manager's attention on those cows likely to return to heat;
2. Breeding plans can be used more efficiently;
3. Non-producing cows can be culled early;
4. Fertility testing of bulls is simplified; and,
5. Diagnosis of reproductive problems is facilitated.

Radioimmunoassay of progesterone from milk samples allows great quantities of data regarding the reproductivity of dairy animals to be easily obtained by the researcher. However, some method of analyzing this data needs to be developed. If the researcher can establish a

"normal" pattern of reproductivity through this analysis then possibly the radioimmunoassay of progesterone will provide a method of monitoring the effects of some of the preceding factors influencing fertility.

MATERIALS AND METHODS

The information used in this study was obtained from two dairy herds, one located at the University of British Columbia South Campus, UBC herd and the other at the Agriculture Canada Research Station at Agassiz, B.C., Agassiz herd.

1. Milk Sampling

Post-milking, whole milk strippings were collected in 200 ml Whirl Pak® bags, using potassium dichromate LACTAB® as the preservative and stored at 4-10°C until analysis was performed. The Agassiz herd was sampled on an every other day basis while the UBC herd was sampled twice a week with sampling beginning approximately six days post-partum and continuing until approximately 60 days post-conception. Samples from the Agassiz herd were either mailed, or brought to the University for analysis.

2. Radioimmunoassay

The samples were analysed for milk progesterone by radioimmunoassay employing a method described by Shelford et al. (1979) which is a modification of the radioimmunoassay technique first described by Heap et al. (1973).

3. Animals - General Management Practices

3.1 UBC Herd

A total of 53 animals were sampled through 54 lactations between February 1978 and June 1979. These animals kept at the south campus

facilities were placed in maternity pens six to ten days prior to expected parturition and remained there until five to ten day post-partum. The cows were then housed in a free stall confinement system with access to a row of 48 Calan Broadbent electronically controlled individual feeders. The animals also had access to an outside paved exercise area, weather permitting.

Feeding took place at approximately 0230 hours and 1300 hours (prior to milking), and consisted of alfalfa cubes (fed ad lib), 14% protein textured concentrate (ratio of 1 grain to 3 milk), and beet pulp pellets (max. 4 kg per day). A few animals also were fed ad lib an experimental ration of corn silage and an 18% protein textured concentrate on a 70 to 30 ratio of forage to grain.

Cows were moved to a holding area at approximately 0330 hours and 1400 hours and milked in an in-line, three stall, high-line parlour, beginning at approximately 0400 hours and 1430 hours.

No set times were established for estrus detection, however, the majority of the heats were noted prior to feeding and milking. The general practice of monitoring estrus behaviour in the herd was that anyone working with the cattle would note any estrus activity, i.e. mounting, increased activity or vaginal discharge, and inform the herdsman, who would act accordingly. Some animals not noted in standing heat during the study were bred on the basis of previously predicted heats as determined from milk progesterone analysis.

Artificial insemination was performed by the herdsman at approximately 1500 hours for those animals noted in heat in the early

morning hours, and at approxiamtely 0830 hours for those animals noted in heat in the previous afternoon and evening. Pregnancy diagnosis by rectal palpation and post-calving reproductive checks were performed at irregular intervals, 30-60 days apart.

When the veterinarian was in attendance for pregnancy diagnosis, all post-partum animals not previously examined were checked to determine the health status of the reproductive tract. Also, any cows showing any abnormal reproductive occurrence, nymphomania, anestrus, abnormal vaginal discharge, etc., were examined, and treatment was initiated when any abnormality was diagnosed. Individual records consisted of dates of calving, estrus, breeding, treatment of reproductive tract and positive rectal palpation of pregnancy by a veterinarian. Health data such as retained placental membranes, metritis, milk fever, ketosis, cystic ovaries (when examined by a veterinarian) and mastitis were also noted.

Throughout the sampling period 12 animals were culled from the herd. Data collected from these animals was used for some of the calculations if appropriate. For example, if early sampling continued up to first estrus but not until conception, the data would be used in calculation of days to first estrus but would not be included in any pregnancy test calculations.

3.2 Agassiz Herd

A total of 110 animals were sampled through 146 lactations from mid-December 1976 to mid-September 1979.

The Agassiz herd was housed in a free stall barn. In contrast to the UBC herd, the Agassiz herd was pastured from May to October on Orchard Grass with approximately 10% White Clover, using a strip grazing system. Corn silage and approximately 2 kg. long hay made up the roughage ration during the winter. The grain ration was fed in the double sawtooth parlour at the rate of 1 kg grain to 3 kg of milk yield in winter and at the rate of 1 kg grain to 4 kg of milk yield in the summer. Depending on other experimental regimes or level of production, additional grain was fed in the parlour.

There were set times for estrus detection in the Agassiz herd. An hour in the late evening, from 2100 to 2200 hours was set aside for observation. As well, the animals were observed at milking and feeding. Similar to the UBC herd, those animals not noted in standing heat during the study, and showing regular heat as predicted by progesterone analysis, may have been bred on days of "expected heat." All breeding was by artificial insemination and animals were palpated for pregnancy at approximately two months gestation, along with any abnormal animals.

Individual information was collected from the record system used for the herd, and was similar to that collected for the UBC herd. Throughout the sampling period the animals from which information was collected were also used for other trials and examinations, some of which involved an early and late breeding regime. For the early bred group, animals were inseminated at the first visible heat following 50 days post-partum, while the late bred group was inseminated following 80

days post-partum (Schneider et al., 1981). This may have affected the results of some of the reproductive statistics determined for the Agassiz herd.

Throughout the sampling period animals were culled from this herd for normal reasons, illness, reproductive problems, etc, as well as a herd reduction. Data collected from these animals was used if appropriate.

4. Cycle Classification

4.1 Cycle Phases

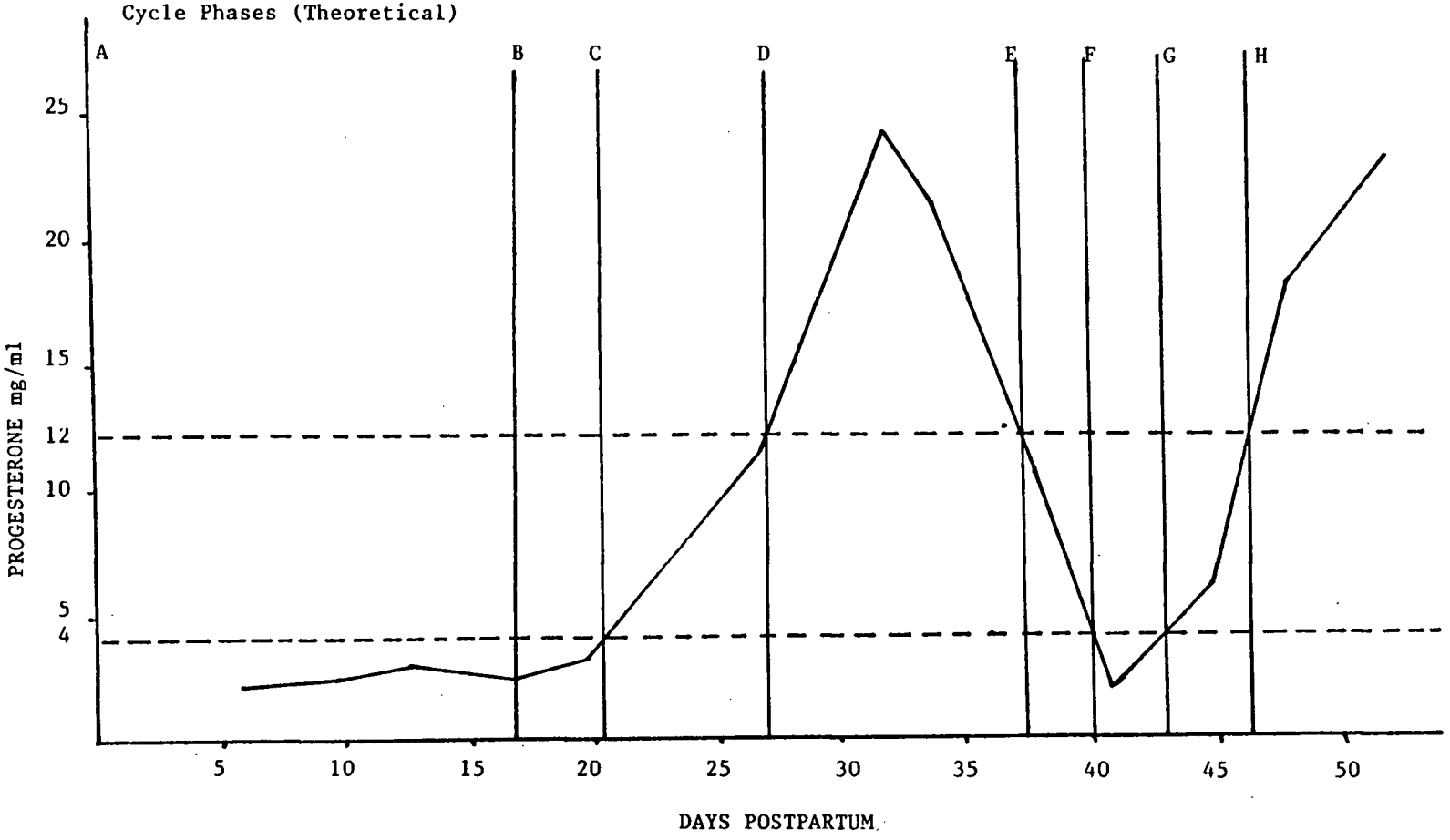
The estrous cycle was classified into phases based on the concentration of progesterone in the milk samples, to aid in identification of different types of cycles. Examples of these phases derived from the following criteria are shown in Fig. 1a representing a theoretical classification and Fig. 1b representing the classification as derived from the data.

Phase 0: Represents the stage of quiescence in the ovaries with respect to luteal activity immediately post-calving, and lasts until the last sample date with the lowest concentration of progesterone before a rise above a concentration of progesterone of 5 ng/ml milk.

Phase 1: Represents the follicular stage of the ovarian cycle when the concentration of progesterone in the sample is less than 4 ng/ml milk.

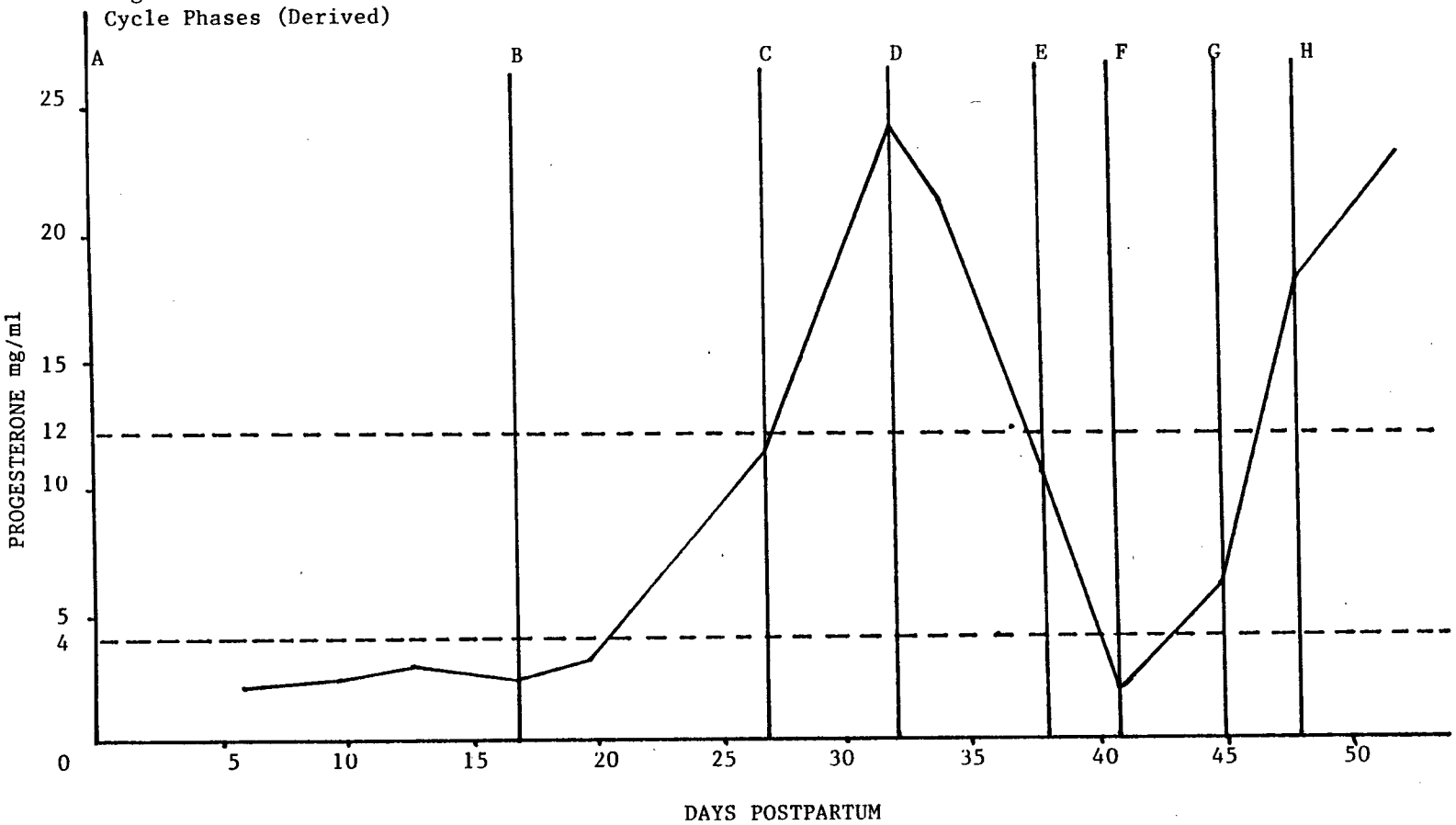
Phase 2: Represents the developing corpus luteum stage after ovulation when the concentration of progesterone is rising from 4 ng/ml

Fig. 1a
Cycle Phases (Theoretical)



Phase	Location	Physiological Stage
0	A → B	Quiescence
1	B → C, F → G	Follicular Stage
2	C → D, G → H	Developing Corpus Luteum Stage
3	D → E	Luteal Stage
4	E → F	Regressing Corpus Luteum Stage

Fig. 1b
Cycle Phases (Derived)



Phase	Location	Physiological Stage
0	A → B	Quiescence
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2	C → D, G → H	Developing Corpus Luteum Stage
3	D → E	Luteal Stage
4	E → F	Regressing Corpus Luteum Stage

milk to 12 ng/ml milk.

Phase 3: Represents the luteal stage when the corpus luteum is fully active and producing progesterone in concentrations in excess of 12 ng/ml milk.

Phase 4: Represents the regressing corpus luteum stage and the maturing of the new follicle, when the concentration of progesterone is declining from 12 ng/ml milk to 4 ng/ml milk.

4.2 Types of Cycles

Once a progesterone concentration indicative of a resumption of luteal activity was established and the cycle was classified into different phases, a method of classifying the cycles into various types was required. Criteria for selection for the various types are given below.

Table 5

Cycle Types

Cycle Types	Descriptive Term
1	Short first cycle
2	Normal first cycle
3	Normal cycle, not type 2, not bred
4	Pregnant cycle
5	Early embryonic abortion
6	Atypical cycles, not bred
7	Normal cycle, bred but not conceiving
8	Atypical cycles with a breeding but not conceiving

Upon observing the graphs of progesterone concentration versus time for a number of animals, two distinct types of first cycles were determined.

Type 1: This was defined as the short first cycle after quiescence,where the sample concentration of progesterone went from less than 4 ng/ml milk to greater than 5 ng/ml milk, and returned to less than 4 ng/ml milk in less than 17 days.

In a study of 690 normal cows, Morrow et al. (1969) found 40% of the cycles were less than 17 or greater than 23 days in length.

Type 2: This was defined as the normal first cycle after quiescence, where the sample concentration of progesterone went from less than 4 ng/ml milk to greater than 12 ng/ml milk, and returned to less than 4 ng/ml milk, in 17 to 25 days. This was essentially a normal cycle.

Type 3: This was a normal cycle subsequent to the first cycle, with the sample concentration rising from less than 4 ng/ml milk to greater than 12 ng/ml milk, and falling to less than 4 ng/ml milk, in 17-25 days, not including Type 2 cycles. A further constraint of not being bred was imposed on these cycles to distinguish them from Type 7 cycles.

Type 4: These cycles represented pregnancy, and the sample concentration had to remain greater than 12 ng/ml milk for 25 days after breeding.

Type 5: This group represented early embryonic abortion, and the sample concentration of progesterone had to remain greater than 12 ng/ml

milk for 25 days after breeding, similar to Type 4, but subsequently fall to less than 4 ng/ml milk.

Type 6: This group represented the atypical cycles, with no breeding.

Type 7: This group was similar to Type 3, a normal cycle, however, it included those animals bred and not conceiving.

Type 8: This group represented those atypical cycles that included a breeding, not leading to conception.

Means, standard deviations, and significance tests, for each cycle phase and cycle type, for both UBC and Agassiz herds were obtained, using the package program UBC SPSS.

4.3 Standard Curves

To develop a standard curve for a normal cycle all those cycles classified as Type 2, the first normal cycles and Type 3, the normal cycles were pooled for each data base.

Day one was determined to be the first sampling date. These cycles were then grouped by cycle length. Means, standard deviations and the number of observations for each group were calculated for each sample date in each group using the UBC Triangular Regression Package.

RESULTS AND DISCUSSION

The results have been presented as the concentration of progesterone in milk, though it should be recognized that the values probably over-estimate the true progesterone concentration because of the occurrence of steroid metabolites in the cow's milk that cross-react with the anti-serum (Heap et al., 1976). Measurements of known amounts of progesterone with this analysis have also shown a tendency to over-estimate low values and under-estimate high values (Heap et al., 1973).

1. Resumption of Luteal Activity

Cycling post-calving was determined to have begun once the concentration of progesterone in the sample had reached a level of more than 5 ng/ml milk. The time from parturition until the last sample date with a concentration of progesterone of less than 5 ng/ml milk was called the stage of quiescence.

Examples of a normal short quiescence of 20 days, and an abnormally long quiescence of 51 days, are given in Figs. 2 and 3 respectively.

From the Agassiz data there were 127 cases of quiescence examined. The mean number of days in quiescence was 19.81 ± 8.854 S.D., with a range of 56 days. A total of 13% were 30 days or more in quiescence.

Of the 127, 12.6% or 16 animals, were recorded as having retained placenta. They had a mean of 25.62 ± 12.41 S.D. days in quiescence,

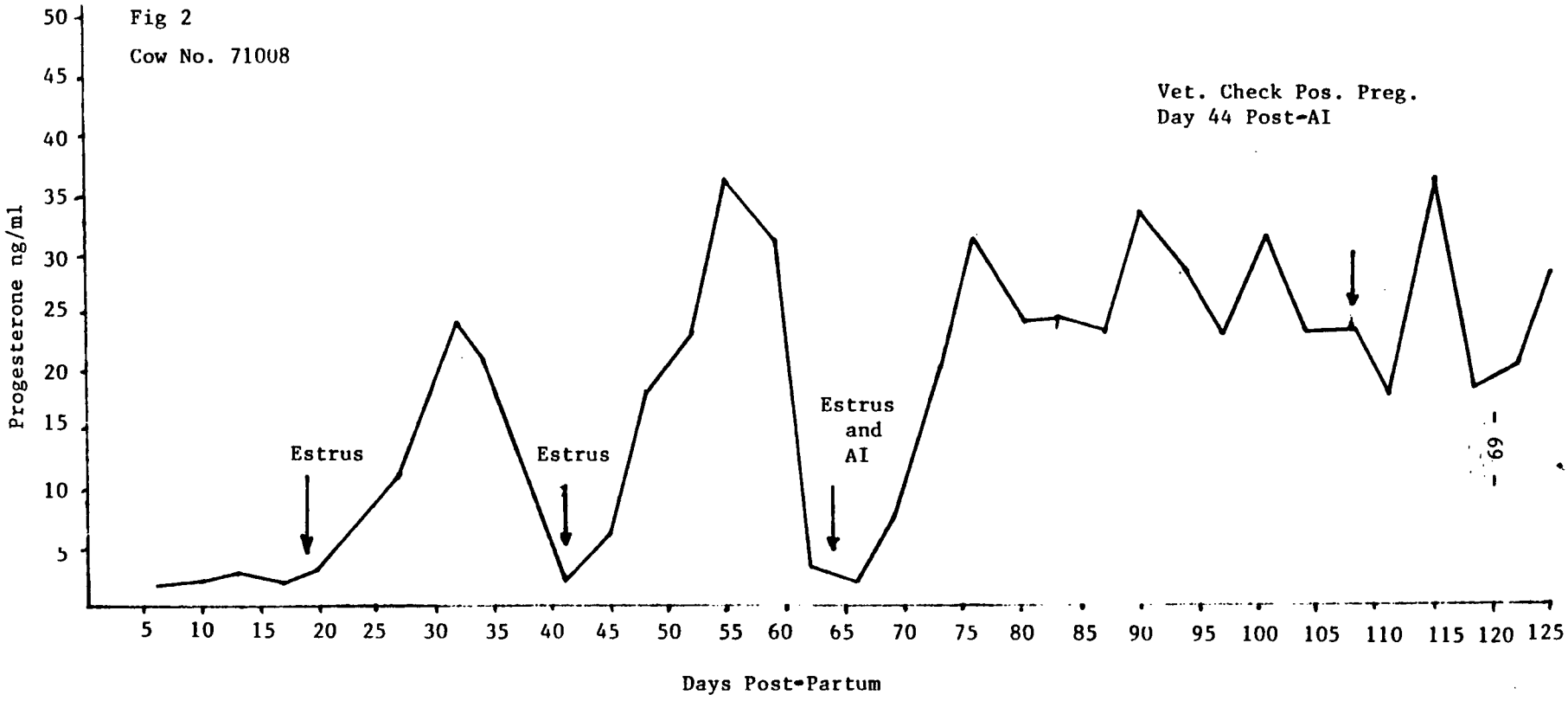
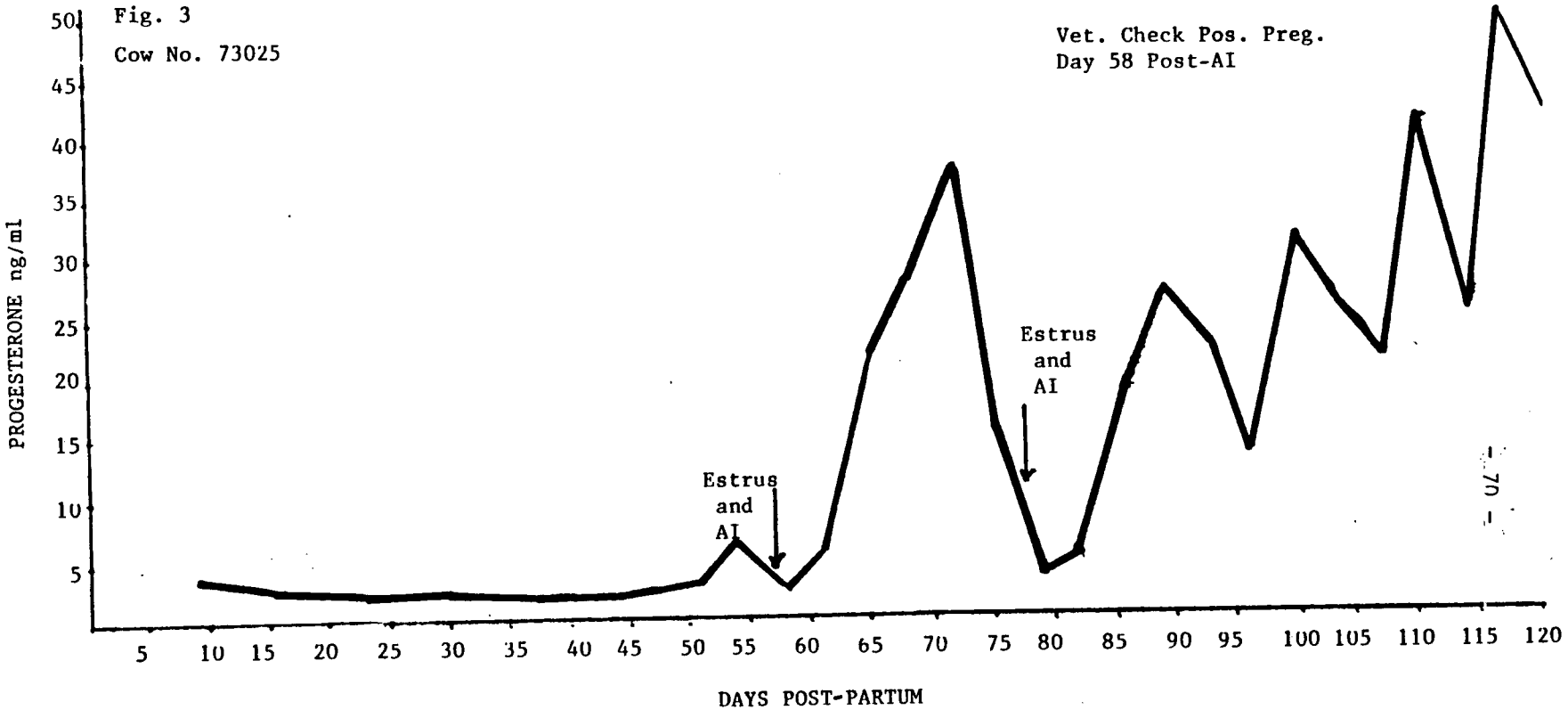


Fig 2
Cow No. 71008
Vet. Check Pos. Preg.
Day 44 Post-AI
Progesterone ng/ml
50
45
40
35
30
25
20
15
10
5
Estrus
Estrus
Estrus
and
AI
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125
Days Post-Partum

Fig. 3

Cow No. 73025



with a range of 50 days. Only 18.7% or three animals were longer than 30 days in quiescence.

For the remaining 111, the mean number of days in the period of quiescence was 18.97 ± 7.95 S.D. with a range of 43 days. Of these 16% or 13 animals were 30 days or more in quiescence.

Examination of the UBC data revealed 54 cases of quiescence lasting for a mean of 21.43 ± 11.84 S.D. days, with a range of 55 days. Of these, 24% or 13 animals were 30 days or longer in quiescence.

Five cases of retained placenta were noted in the UBC data. They showed a surprisingly short quiescence, with a mean number of days in quiescence of 10.80 ± 6.42 S.D. and a range of 16 days.

Treatment for retained placenta in the UBC herd consisted of an attempt at manual removal of the placenta if not too firmly attached, followed by the insertion of antibiotic boluses. Following removal of placenta, if any further abnormal discharges from these animals were noted, they received a uterine infusion of an antibiotic solution.

For the remaining 49 without retained placenta the mean number of days in quiescence was 21.86 ± 11.97 S.D., with a range of 55 days. Of these 25% or 13 animals were 30 days or more in quiescence.

Out of 54 cases of quiescence examined from the UBC data, only two animals or 3.7% remained inactive for more than 50 days. Only one of 127 cases of quiescence in the Agassiz data was greater than 50 days.

These results are similar to those found by van de Wiel and co-workers in 1979 but do not match those of Bulman and Wood (1980) who found 4.9% of 533 dairy cows took longer than 50 days post-partum to

resume cycling.

The greatly reduced time in quiescence for those animals in the UBC herd treated for retained placenta is a mystery. One would expect that a reproductive problem of this type would extend the time in quiescence as was the case in the Agassiz data.

One possible explanation for this reduction is that the frequent manual examination of the uterus, the uterine infusion or the combination may have had a stimulating effect on the ovaries.

2. Cycle Types

By the end of the sequential sampling time period for Agassiz's every second day sampling and UBC's twice weekly sampling, over 13,000 samples and information were collected, organized and categorized into cycles phases and cycle types as described in Materials and Methods.

The number of observations, means, and standard deviations, for progesterone concentration (ng/ml) and days in the phase, for Phases 1 through 4 of all cycle types for the Agassiz data and the UBC data, are given in Tables 6-13. They were grouped into homogenous subsets derived from the Student-Newman-Keuls procedure using the package program UBC SPSS.

From the study of many of the profiles, it was apparent there was a great deal of variation in the first rise of progesterone post-partum and the associated first cycle. Many of the profiles showed a small transient rise in progesterone before a normal cycle while others indicated that the first rise continued into a normal type of cycle.

Table 6

Phase 1 - UBC Data
Follicular Stage

Cycle Type	Number of Observations	(ng/ml) Mean Progesterone ± St. Dev.	Mean No. of Days ± St. Dev.
1. Short First Cycle	18	2.49 ± .37ab	5.83 ± 2.79^{AB}
2. Normal First Cycle	20	2.32 ± .35a	6.80 ± 2.80^{AB}
3. Normal Cycle Not Type 2 Not Bred	65	2.62 ± .58ab	5.54 ± 2.44ab
4. Pregnant Cycle	39	2.77 ± .62ab	5.18 ± 2.04ab
5. Early Embryonic Abortion	2	2.65 ± .21ab	5.00 ± 2.83ab
6. Atypical Cycle Not Bred	51	2.65 ± .59ab	15.47 ± 24.89b
7. Normal Cycle Bred not Conceived	27	2.42 ± .65ab	4.93 ± 2.60a
8. Atypical Cycle With Breeding	12	3.00 ± .66b	10.25 ± 8.30ab
Total	234	2.61 ± .58	7.94 ± 12.56

a,b Denote Homogeneous subsets derived from the Student-Newman-Keuls Procedure at $P < 0.05$.

Table 7

Phase 2 - UBC Data
Developing Corpus Luteum Stage

Cycle Type	Number of Observations	(ng/ml) Mean Progesterone ± St. Dev.	Mean No. of Days ± St. Dev.
1. Short First Cycle	17	5.90 ± 1.43	4.47 ± 2.29
2. Normal First Cycle	9	7.26 ± 2.03	3.78 ± 1.39
3. Normal Cycle Not Type 2 Not Bred	45	7.14 ± 2.00	3.73 ± 1.01
4. Pregnant Cycle	23	6.69 ± 1.99	4.43 ± 2.95
5. Early Embryonic Abortion	1	8.70	4.00
6. Atypical Cycle Not Bred	31	6.54 ± 2.38	5.58 ± 4.71
7. Normal Cycle Bred not Conceived	21	7.30 ± 2.46	4.00 ± 1.09
8. Atypical Cycle With Breeding	10	8.30 ± 2.35	5.10 ± 3.35
Total	157	6.93 ± 2.15	4.41 + 2.77

Table 8

Phase 3 - UBC Data
Luteal Stage

Cycle Type	Number of Observations	(ng/ml) Mean Progesterone ± St. Dev.	Mean No. of Days ± St. Dev.
1. Short First Cycle	11	18.78 ± 3.88a	6.18 ± 3.54a
2. Normal First Cycle	20	28.20 ± 8.65ab	10.50 ± 3.85a
3. Normal Cycle Not Type 2 Not Bred	68	32.21 ± 9.46a	13.57 ± 3.03a
4. Pregnant Cycle	46	42.14 ± 7.59b	80.87 ± 27.64b
5. Early Embryonic Abortion	3	37.77 ± 13.55ab	27.00 ± 7.20a
6. Atypical Cycle Not Bred	58	32.19 ± 12.13a	15.5 ± 13.53a
7. Normal Cycle Bred not Conceived	27	33.48 ± 9.72a	12.67 ± 2.83a
8. Atypical Cycle With Breeding	11	34.81 ± 12.19ab	22.36 ± 25.12a
Total	244	33.47 ± 11.02	26.59 ± 30.21

a,b Denote Homogeneous subsets derived from the Student-Newman-Keuls Procedure at $P < 0.05$.

Table 9

Phase 4 - UBC Data
Regressing Corpus Luteum Stage

Cycle Type	Number of Observations	(ng/ml) Mean Progesterone ± St. Dev.	Mean No. of Days ± St. Dev.
1. Short First Cycle	1	4.60	3.00
2. Normal First Cycle	8	6.96 ± 2.24	4.75 ± 2.87
3. Normal Cycle Not Type 2 Not Bred	15	6.62 ± 2.26	4.47 ± 2.26
4. Pregnant Cycle	3	10.00 ± 2.26	3.00 ± 1.00
5. Early Embryonic Abortion	0		
6. Atypical Cycle Not Bred	29	6.92 ± 2.49	6.93 ± 5.47
7. Normal Cycle Bred not Conceived	7	6.00 ± 1.73	3.43 ± .53
8. Atypical Cycle With Breeding	5	8.70 ± 1.32	7.20 ± 6.22
Total	68	7.00 ± 2.36	5.56 ± 4.36

Table 10

Phase 1 - Agassiz Data
Follicular Stage

Cycle Type	Number of Observations	(ng/ml) Mean Progesterone ± St. Dev.	Mean No. of Days ± St. Dev.
1. Short First Cycle	58	2.35 ± .36	2.88 ± 1.38a
2. Normal First Cycle	26	2.34 ± .32	3.08 ± 1.16a
3. Normal Cycle Not Type 2 Not Bred	173	2.35 ± .45	5.72 ± 1.85a
4. Pregnant Cycle	89	2.39 + .46	5.81 ± 1.95a
5. Early Embryonic Abortion	4	2.50 ± .49	7.00 ± 2.58ab
6. Atypical Cycle Not Bred	86	2.39 ± .66	6.94 ± 9.71a
7. Normal Cycle Bred not Conceived	98	2.43 ± 1.12	5.95 ± 1.64a
8. Atypical Cycle With Breeding	68	2.31 ± .40	16.28 ± 30.26b
Total	602	2.37 ± .62	6.76 ± 11.42

a,b Denote Homogeneous subsets derived from the Student-Newman-Keuls Procedure at $P < 0.05$.

Table 11

Phase 2 - Agassiz Data
Developing Corpus Luteum Stage

Cycle Type	Number of Observations	(ng/ml) Mean Progesterone ± St. Dev.	Mean No. of Days ± St. Dev.
1. Short First Cycle	65	6.55 ± 1.92	3.72 ± 2.39
2. Normal First Cycle	23	7.96 ± 1.99	2.78 ± 1.16
3. Normal Cycle Not Type 2 Not Bred	154	7.28 ± 1.97	2.96 ± 1.52
4. Pregnant Cycle	77	7.00 ± 1.89	2.65 ± .99
5. Early Embryonic Abortion	4	8.65 ± 1.29	2.00 ± .00
6. Atypical Cycle Not Bred	75	6.31 ± 1.87	3.52 ± 2.61
7. Normal Cycle Bred not Conceived	89	7.06 ± 1.91	2.88 ± 1.28
8. Atypical Cycle With Breeding	48	6.84 ± 1.85	3.39 ± 2.29
Total	535	6.98 ± 1.95	3.09 ± 1.83

Table 12

Phase 3 - Agassiz Data
Luteal Stage

Cycle Type	Number of Observations	(ng/ml) Mean Progesterone ± St. Dev.	Mean No. of Days ± St. Dev.
1. Short First Cycle	55	24.64 ± 8.55ab	6.25 ± 3.63a
2. Normal First Cycle	26	29.38 ± 8.84bc	13.54 ± 2.79a
3. Normal Cycle Not Type 2 Not Bred	164	32.05 ± 8.69c	12.32 ± 2.95a
4. Pregnant Cycle	96	37.57 ± 7.11d	50.86 ± 39.69b
5. Early Embryonic Abortion	4	43.15 ± 3.32d	37.00 ± 11.01ab
6. Atypical Cycle Not Bred	133	23.25 ± 8.93a	9.22 ± 12.42a
7. Normal Cycle Bred not Conceived	107	30.13 ± 8.28c	12.74 ± 2.97a
8. Atypical Cycle With Breeding	70	25.67 ± 10.21ab	19.56 ± 38.47a
Total	655	29.42 ± 9.87	17.87 ± 24.96

a,b,c,d Denote Homogeneous subsets derived from the Student-Newman-Keuls Procedure at $P < 0.05$.

Table 13

Phase 4 - Agassiz Data
Regressing Corpus Luteum Stage

Cycle Type	Number of Observations	(ng/ml) Mean Progesterone ± St. Dev.	Mean No. of Days ± St. Dev.
1. Short First Cycle	26	7.30 ± 2.21	2.61 ± 1.47a
2. Normal First Cycle	6	6.70 ± 1.38	4.00 ± 4.00a
3. Normal Cycle Not Type 2 Not Bred	45	7.28 ± 2.49	2.04 ± .29a
4. Pregnant Cycle	1	8.50	51.00b
5. Early Embryonic Abortion	1	4.10	2.00a
6. Atypical Cycle Not Bred	77	8.75 ± 2.21	3.67 ± 10.17a
7. Normal Cycle Bred not Conceived	35	6.75 ± 2.15	2.26 ± 1.01a
8. Atypical Cycle With Breeding	47	9.55 ± 2.50	3.00 ± 3.25a
Total	238	8.11 ± 2.51	3.11 ± 6.79

a,b Denote Homogeneous subsets derived from the Student-Newman-Keuls Procedure at $P < 0.05$.

Robertson (1972) using plasma progesterone assumed that some of the early post-partum progesterone peaks could have been associated with follicle luteinization rather than with true ovulation.

Caudle et al. (1982) found that nine of 21 cows in their study returned to ovarian function with short elevations in milk progesterone. Unable to determine if the source of progesterone was corpus luteum or luteinized follicles, they postulated the control mechanism for progesterone secretion operated differently during the puerperal period if a different cell source is responsible for progesterone secretion.

Examples of these two types of first cycles can be found in Figs. 2 and 3. Figure 2, cow 71008 shows a dramatic initial rise of progesterone with the cycle lasting a normal 21 days. This would be classified as Type 2. In contrast, Fig. 3, the graph of the progesterone cycle for cow 73025 illustrates the short first cycle Type 1 which may be associated with follicle luteinization rather than true ovulation, and may in fact act as an alternate starter mechanism as the following cycle from day 57 to day 79 appears to be a normal cycle. A successful breeding is noted on day 78 post-partum for this animal and is illustrated by a rising progesterone level.

Examining Table 6, Phase 1, the follicular stage of the UBC data we find that 18 of 38 cows or 47% returned to ovarian function with a short first cycle Type 1. Table 10, Phase 1 of the Agassiz data shows 58 of 84 cows or 69% returned to ovarian function with a Type 1 cycle.

Following these first cycles through the remaining phases of the cycle we find that during Phase 2, the developing corpus luteum stage,

there were only 26 first cycles classified in the UBC data as shown in Table 7. Of these 17 cycles or 65% were classified as being Type 1. Table 11 Phase 2 of the Agassiz data shows 88 first cycle classifications. Of these 65 cycles or close to 74% were identified as being the short first cycle Type 1 variety.

Thirty-one first cycles classifications were identified in Phase 3, the luteal stage from the UBC data as shown in Table 8. Of these 11 cycles or 35% were identified as Type 1. Table 12 Phase 3 of the Agassiz data showed 81 first cycle classifications of which 55 cycles or 68% were classified as Type 1.

For Phase 4, the regressing corpus luteum stage only one of nine first cycles or 11% fell into the classification for short first cycle Type 1 in the UBC data as shown in Table 9. Twenty-six of 32 first cycles or 81% of the first cycles found in the Agassiz data to be in Phase 4 were identified as being Type 1 as shown in Table 13.

It appears as though the reduced frequency of sampling in the UBC data has led to more of the first cycles being classed as Type 2 normal first cycles rather than as Type 1 short first cycles compared to the results of the Agassiz data. The results of the Agassiz data show that the number of animals which return to ovarian function with a short first cycle out number those that return with a normal length first cycle.

Another trend noted in the comparison of the results of the UBC sampling regime with that of the Agassiz sampling procedure was that days in Phase 1 for the Agassiz data was approximately one half that

found for the UBC data, with the values being 2.88 ± 1.38 days, n = 58; 3.08 ± 1.16 days, n = 26 vs 5.83 ± 2.79 days, n = 18; 6.80 ± 2.80 days, n = 20 for Agassiz Type 1 and Type 2 vs UBC Type 1 and Type 2 respectively.

The length of time in Phase 2 was also shorter in the Agassiz data for these cycle types. This trend was not evident during Phase 3 when the number of days in Phase 3 for the Type 2 cycles was less in the UBC data compared to the Agassiz data. Length of time spent in Phase 4 for these cycle types were very similar for both data sets.

There were no significant differences noted between cycle Types 1 and cycle Type 2 for days in phases for both the UBC data and the Agassiz data. This was also the case for mean progesterone concentration in the phase except that during the early stages of progesterone production, Phase 2, the developing corpus luteum stage, the short cycle type tended to reach a lower level in both the Agassiz data and the UBC data. During Phase 3, the luteal stage, the same trend was noted with the addition that the short first cycle tended to occupy this period for fewer days. In fact, days in Phase 3 was shortest for Type 1 cycles when compared to all other types. It was expected from the criteria of selection that Type 1 short first cycles would be the shortest. It is interesting to note that it occurs in the active luteal stage of the cycle or Phase 3.

This tendency of reduced progesterone concentration and reduced days in the luteal stage tend to further emphasize the postulation by Caudle and co-workers in 1982, that the control mechanism for progesterone secretion operated differently during the puerperal period

if a different cell source is responsible for progesterone secretion. Perhaps the normal first cycle, Type 2 source is the corpus luteum, while the short first cycle, Type 1 source is luteinized follicles.

Only a total of nine cases were identified as Phase 4 first cycles compared to 38 cases of first cycle Phase 1, 26 cases of first cycle Phase 2 and 31 cases of first cycle Phase 3 in the UBC data. The Agassiz data with every other day sampling showed only a few more Phase 4 first cycles, with a total of 32 cases, compared to 84 cases of first cycle Phase 1, 88 cases of first cycle Phase 2 and 81 cases of first cycle Phase 3.

When profiles of the animals are examined, we find that frequently there is a drastic drop in the progesterone concentration for both the sampling regimes, with many samples going directly from a very high level to a very low level with no intermediate concentration noted.

In all, only a total of 68 Phase 4 observations were made compared to 234 Phase 1 observations in the UBC data. With the more frequent sampling of the Agassiz data, still only 238 Phase 4 observations were made compared to 602 Phase 1 observations.

It is apparent therefore that the regressing corpus luteum stage or Phase 4 of the reproductive cycle as defined in the Materials and Methods is very precipitous. Less than half of the cycles examined in either the every second day sampling of the Agassiz data or the twice weekly sampling of the UBC data showed samples with concentrations of progesterone in the range of 12 ng/ml declining to 4 ng/ml.

Perhaps a more frequent sampling regime during this phase of the cycle is required to more accurately determine the beginning of the

regression of the corpus luteum and the maturation of the developing follicle.

One would expect that there would be little difference noted between cycle Type 2 the normal first cycle, and Type 3 the normal cycle not bred, as the criteria for their selection differed only in that cycle Type 2 was a first cycle noted. This was the case with both the UBC data and the Agassiz data with the two cycle types showing similar means for all phases of the cycle.

Normal cycles with a breeding not resulting in a conception, Type 7, were compared with cycles with a successful breeding, Type 4. Phase 1, representing ovulation was similar for both cycle types in the UBC data as well as the Agassiz data. There was no significant difference in the rising phase of progesterone production representing the formation of the corpus luteum between the two cycles types in the two data bases.

A significant difference however was found in Phase 3, representing the fully functional corpus luteum stage. In both the UBC data, and the Agassiz data, cycle Type 4 representing pregnancy reached a greater concentration of progesterone, and maintained it longer. Again, this would be expected from the criteria of selection, as the requirement for cycle Type 4 stated that the concentration of progesterone must remain greater than 12 ng/ml for a minimum of 25 days, while the requirements for cycle Type 7 were similar to the normal cycle but also include a breeding not leading to conception.

Cycles leading to conception, Type 4, were compared to those

cycles leading to early embryonic abortion, Type 5. These cycle types were similar in criteria for selection except that after a minimum of 25 days in Phase 3 the luteal stage, Type 5 cycles would subsequently show a drop in progesterone to less than 4 ng/ml.

As would be expected, there were few observations in the Type 5 cycle category representing early embryonic abortion. In the UBC data the three observations noted for cycle Type 5, Phase 3 reached similar concentration of progesterone as Type 4 cycles, but did maintain that level for a significantly shorter time. This difference was not noted in the Agassiz data because of the earlier cut-off date of sampling post-conception. The Agassiz data tended to cut-off closer to 60 days post-conception, while in many cases the UBC cows were sampled longer after conception.

Cycle Types 6 and 8 were used to classify cycles not fitting into the criteria of the normal cycles such as Type 2, Type 3, Type 7; the criteria of the cycles leading to conception and holding pregnancy, Type 4, and the cycles leading to early embryonic abortion, Type 5, and of course, the criteria of the first short cycle Type 1.

Cycle types 6 and 8 were, therefore, the catch all for those atypical cycles representing reproductive problems, such as cysts of either the corpus luteum or the follicule. The distinction between them was to identify those atypical cycles which also included a breeding, as represented by Type 8.

Phase 3, the fully active luteal stage of the reproductive cycle, showed the greatest number of observations in both the UBC and the

Agassiz data. Of a total of 655 Phase 3 observations in the Agassiz data, approximately 31%, or 203 observations were classified as Type 6 or Type 8, with 34.48% of the atypical cycles containing a breeding, Type 8. Of the 244 Phase 3 observations in the UBC data, 28.28% or 69 observations were classified as Type 6 or Type 8. Of these only 15.94% contained a breeding.

When normal cycles without a breeding, Type 3, were compared to the atypical cycles without a breeding, Type 6, the mean progesterone concentration levels were quite similar for all phases except during the luteal stage, Phase 3 of the Agassiz data in which the atypical cycles without a breeding reached a significantly lower concentration. The mean number of days in the various phases were not significantly different between these two types of cycles.

Comparing cycle Type 6 and Type 8, a significant difference was found in Phase 1 in the Agassiz data. Cycle Type 8, the atypical cycles containing a breeding, remained in Phase 1 significantly longer than did cycle Type 6, the remaining atypical cycles. This would be representative of those cycles containing a follicular cyst, which would prevent development of a corpus luteum, and therefore maintain a low progesterone concentration over a longer period of time. These animals probably exhibited signs of estrus more frequently than those of Type 6, and therefore would lead to their being bred. The delay in ovulation, or the development of a follicular cyst could not have been predicted by the herdsman during insemination, and therefore the number of animals in this category of atypical cycles with breeding is in no way a reflection of the competance of the herdsman or the inseminator.

This difference was not significant in the UBC data. There were no significant differences noted between the two types, Type 6 and Type 8, in the remaining phases of the Agassiz data, or in the UBC data.

When the UBC data was examined the comparison of cycle Type 6, the remaining atypical cycles with cycle Type 7, the normal cycles with breeding showed that the atypical cycles remained significantly longer in the follicular stage of the cycle. This difference was not significant when the comparison was made between Type 7, the normal cycles with breeding and Type 8, the atypical cycles with breeding, however, the mean number of days in phase 1 was less for Type 7.

In the Agassiz data, however this difference was significant between Type 7 and Type 8, with Type 7 remaining in Phase 1, the follicular stage a significantly shorter time, while there were no significant differences between Type 6 and Type 7.

The criteria selected for classifying the reproductive cycle into phases on the basis of progesterone concentration, and the classification of cycle types, does provide a means of handling the large amounts of data which can be easily obtained from milk samples.

A modification of the criteria for selection of Phase 4, the regressing corpus luteum stage, or a modification of sampling regime or both, may provide a better picture of what is happening during this stage of the reproductive cycle.

Since manual examinations per rectum are very subjective and are known to affect the activity of the reproductive tract (Bulman and Lamming 1977), the radioimmunoassay of progesterone from sequentially

sampled dairy cows may be used to monitor the fertility of a dairy herd.

3. Standard Curves

Using the cycle classifications described in Materials and Methods, cycles classified as normal, i.e., with the sample concentration of progesterone rising from less than 4 ng per ml milk to greater than 12 ng per ml milk and subsequently declining to less than 4 ng per ml milk in 17 to 25 days, were grouped according to cycle length, with the first sample designated as day one. Means, standard deviations and the number of observations for each sampling date calculated for each group independently are given in Tables 14 to 21. Profiles of mean progesterone concentration and standard deviations vs. time for each cycle length group are given in Fig. 4 to 7.

Standard deviations for mean progesterone concentration on day one of the cycles, around ovulation were generally smaller than those for sampling dates later when the concentration of progesterone was greater than 5 ng/ml milk, representing luteal activity.

There was a great deal of variation in progesterone concentration during the luteal stage of the "normal" reproductive cycle. This increased variation at higher progesterone concentrations was due mainly to the method of measurement as mentioned in the Literature Review.

As noted earlier in the discussion of the classification of the cycle into the four phases and subsequently into various cycle types, here once again we find evidence that with the sampling regimes used there was a lack of sampling during the fourth phase during the stage of

Table 14

UBC "Normal" 15 Day Cycles

Sample Day	Mean Progesterone Concentration ng/ml	Standard Deviation	Number of Observations
1	2.57	0.61	14
4	10.88	10.99	6
5	14.75	8.77	8
8	24.65	16.03	14
11	29.68	14.81	6
12	28.41	10.75	8
15	24.66	13.68	14

Table 15

UBC "Normal" 18 Day Cycles

Sample Day	Mean Progesterone Concentration ng/ml	Standard Deviation	Number of Observations
1	2.41	0.65	27
4	6.27	5.95	27
8	25.13	16.97	26
11	28.29	14.31	26
15	32.36	14.01	25
18	28.70	14.49	27

Table 16

UBC "Normal" 19 Day Cycles

Sample Day	Mean Progesterone Concentration ng/ml	Standard Deviation	Number of Observations
1	2.51	0.43	16
5	5.88	4.80	16
8	20.52	15.71	16
12	35.22	11.10	15
15	33.77	13.79	15
19	26.98	11.07	16

Table 17

UBC "Normal" 22 Day Cycles

Sample Day	Mean Progesterone Concentration ng/ml	Standard Deviation	Number of Observations
1	2.74	0.83	31
4	2.75	0.72	13
5	4.14	2.66	16
8	14.40	11.52	29
11	24.14	14.57	14
12	28.12	14.53	15
15	38.10	12.29	29
18	30.71	13.04	14
19	38.43	10.85	14
22	24.89	14.52	30

Table 18

Agassiz "Normal" 17 Day Cycles

Sample Day	Mean Progesterone Concentration ng/ml	Standard Deviation	Number of Observations
1	2.26	0.63	27
3	4.43	7.35	25
5	9.74	6.05	26
7	16.17	11.13	26
9	24.65	13.61	26
11	29.22	14.88	26
13	31.00	14.71	25
15	32.55	12.54	26
17	28.87	14.83	26

Table 19

Agassiz "Normal" 19 Day Cycles

Sample Day	Mean Progesterone Concentration ng/ml	Standard Deviation	Number of Observations
1	2.82	3.62	74
3	2.67	1.97	71
5	6.14	6.52	69
7	14.18	10.98	71
9	22.53	12.35	68
11	29.77	12.81	71
13	34.86	13.42	68
15	37.76	12.26	71
17	38.49	12.19	70
19	29.14	15.75	71

Table 20

Agassiz "Normal" 21 Day Cycles

Sample Day	Mean Progesterone Concentration ng/ml	Standard Deviation	Number of Observations
1	2.48	0.77	95
3	2.17	1.09	93
5	3.55	3.31	93
7	8.23	7.17	92
9	18.09	12.38	92
11	26.99	13.70	94
13	29.93	13.50	92
15	33.42	12.69	92
17	34.92	12.66	93
19	35.42	12.29	92
21	21.37	13.98	93

Table 21

Agassiz "Normal" 23 Day Cycles

Sample Day	Mean Progesterone Concentration ng/ml	Standard Deviation	Number of Observations
1	2.75	0.67	43
3	2.18	0.55	42
5	3.09	1.96	43
7	7.01	5.22	43
9	15.14	8.74	42
11	24.60	10.31	42
13	30.70	12.45	43
15	33.02	12.89	43
17	36.19	12.55	42
19	35.86	12.59	42
21	33.79	12.82	42
23	18.07	13.87	43

Fig 4 - UBC NORMAL CYCLES

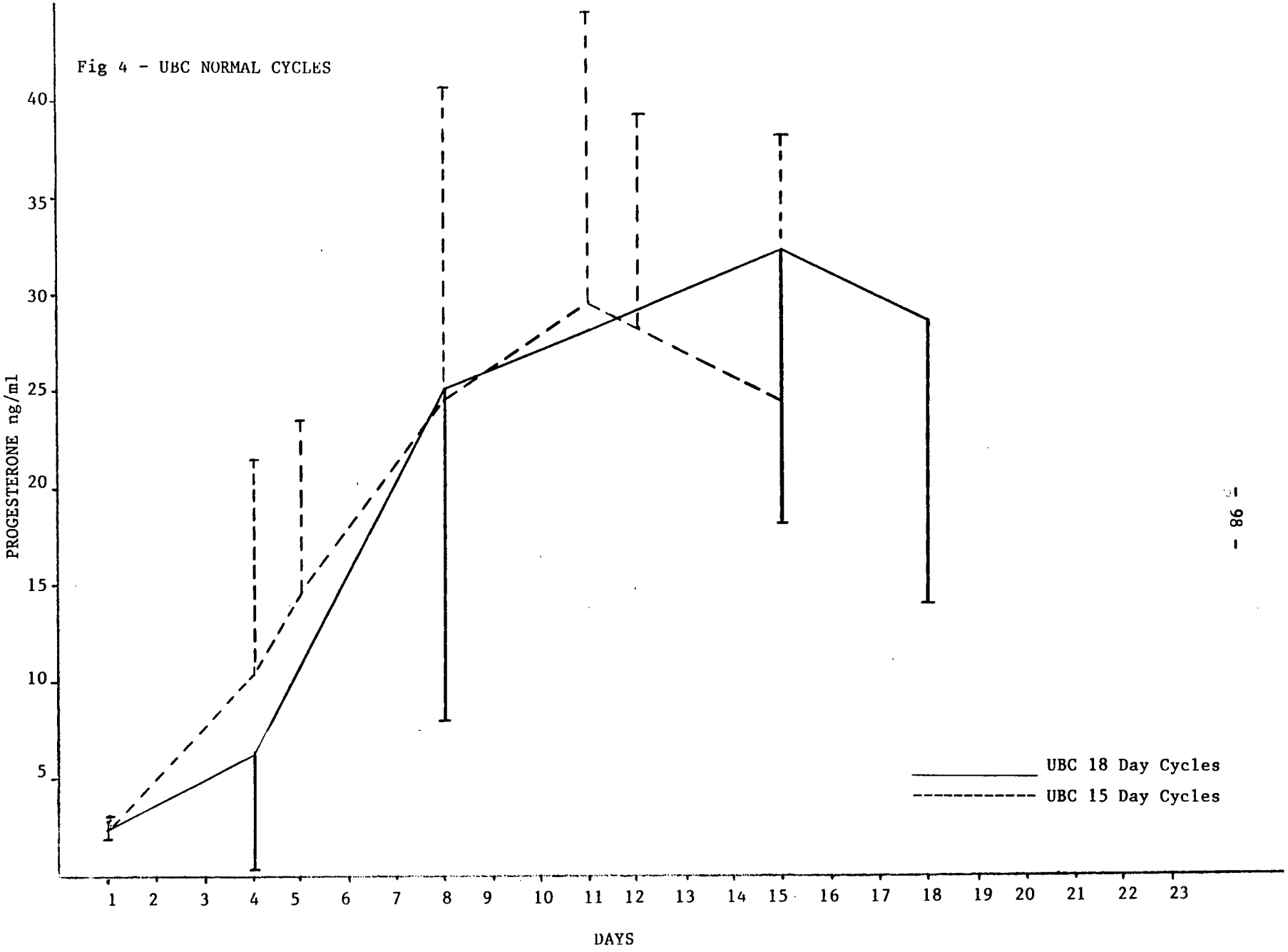


Fig 5 - UBC NORMAL CYCLES

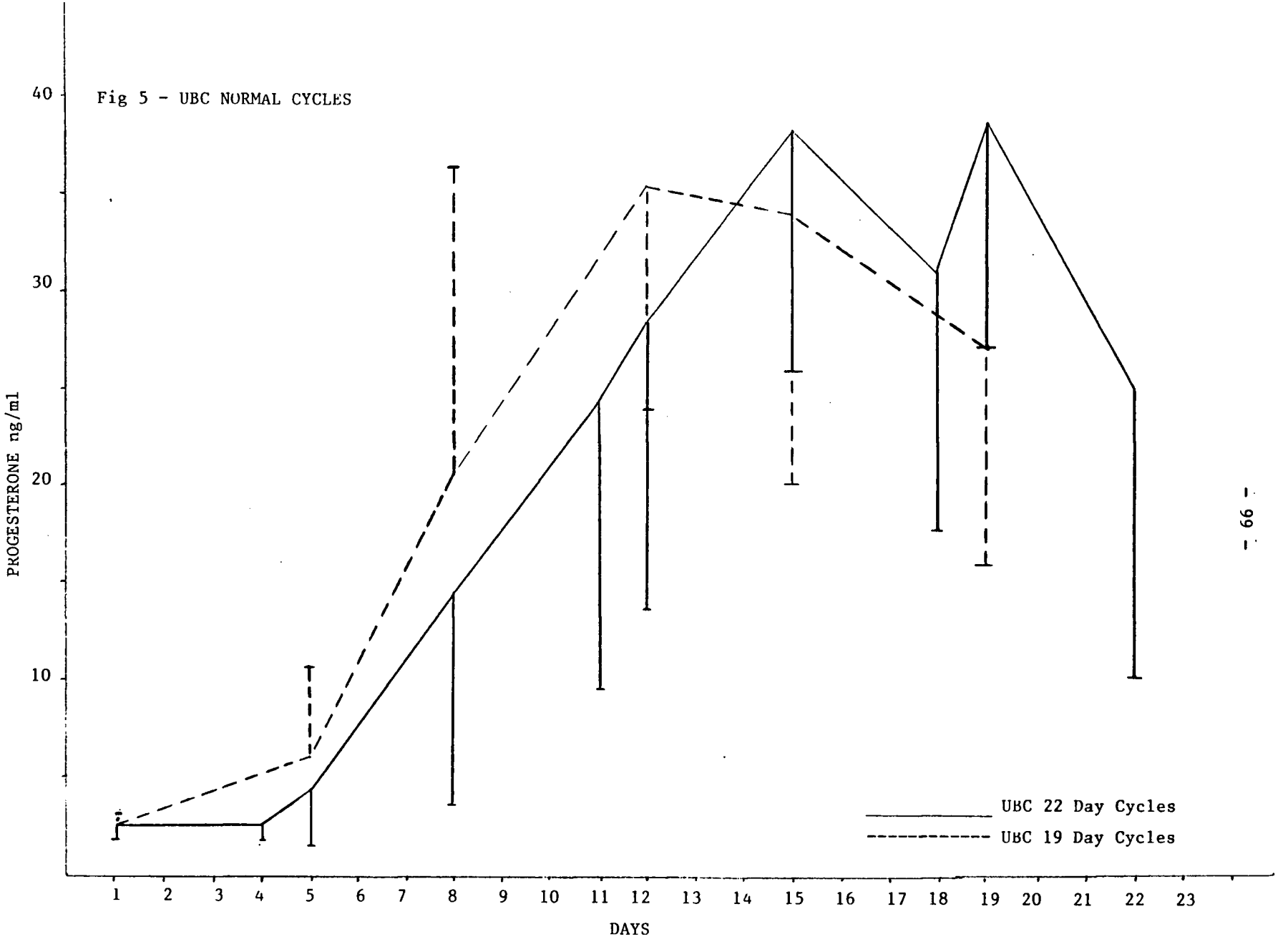


Fig 6 - AGASSIZ NORMAL CYCLES

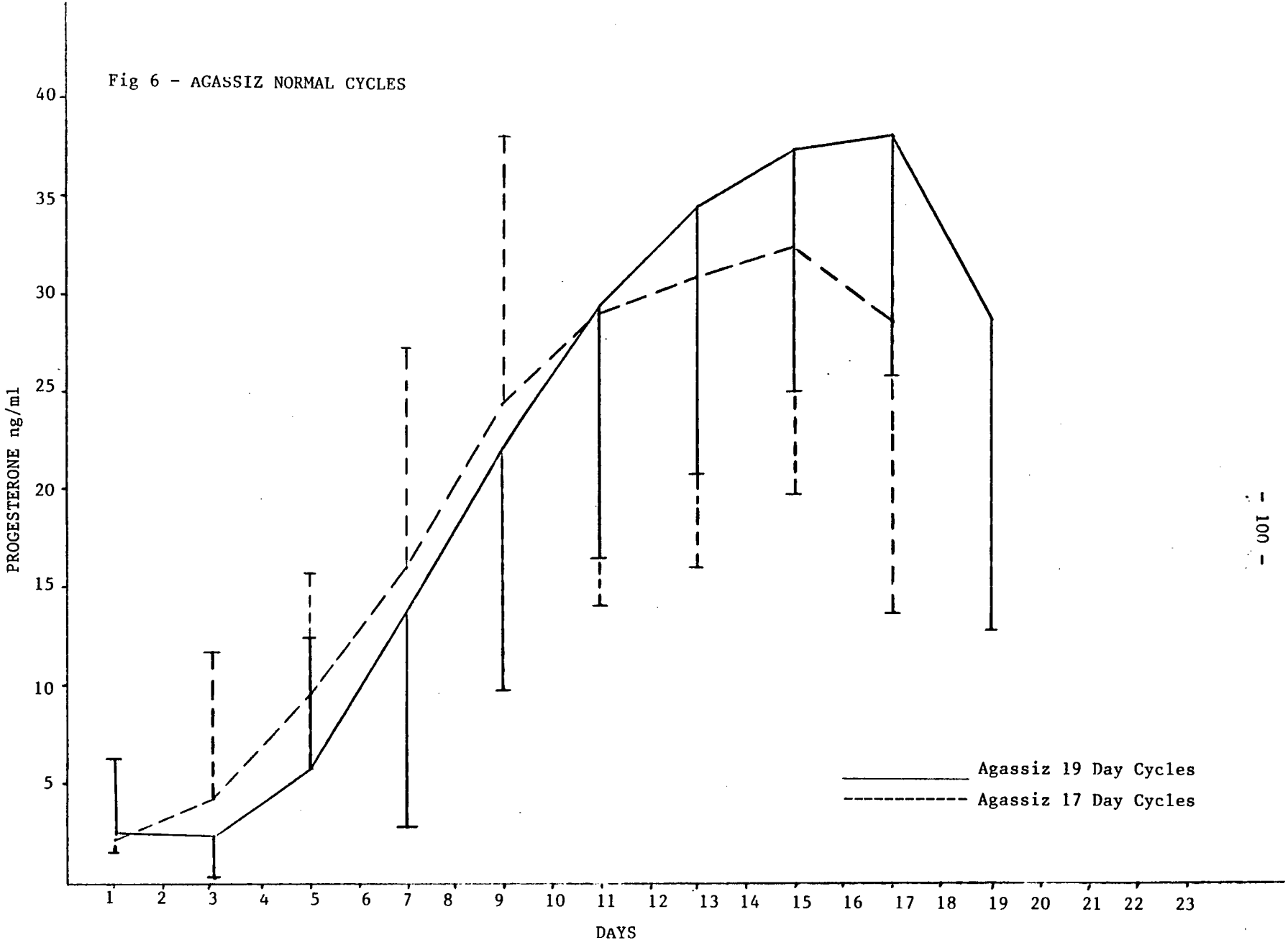
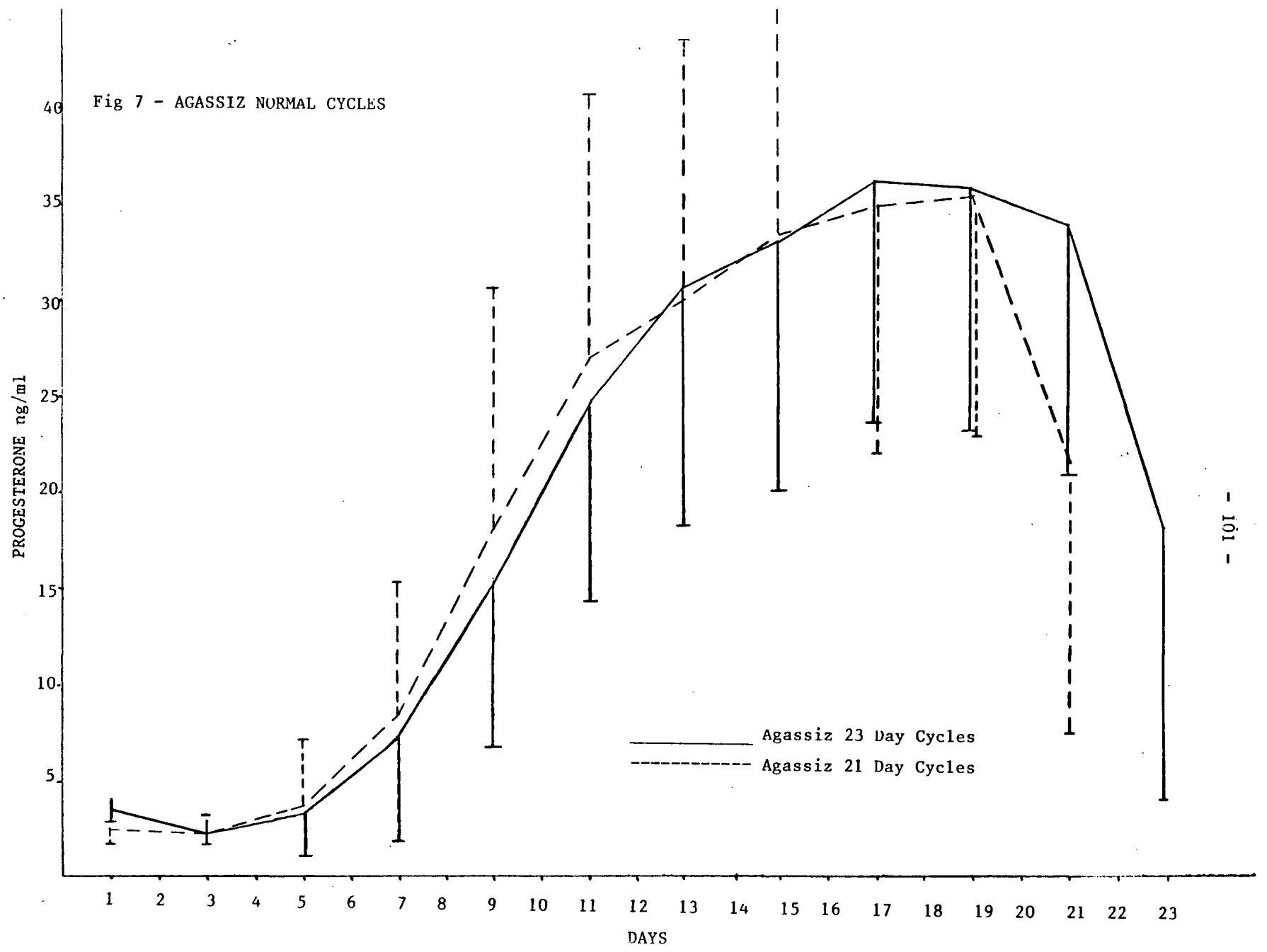


Fig 7 - AGASSIZ NORMAL CYCLES



the regressing corpus luteum and developing follicle when the progesterone concentration should be falling from a value of 12 ng/ml milk or greater to a concentration of progesterone of around 4 ng/ml milk.

This fall from a high concentration, representing luteal activity to a very low value indicative of ovulation therefore must be very precipitous.

With the classification of the cycles as defined, we find that in those cycles of longer duration, such as the UBC "Normal" 22 day cycles, Fig. 5, and the Agassiz "Normal" 23 day cycles, Fig. 7, the profile of the "Normal" cycle does not necessarily begin with day one representing the nadir of progesterone concentration and consequently ovulation.

When the data for normal cycles of various lengths are combined for each data base as in Fig. 8 and 9, we find that the profiles are quite similar, with graphs of increasing cycle length being shifted slightly to the right.

Much of the increase in cycle length may be attributed to increased time spent at a low concentration of progesterone. Duration of this period may be correlated to cycle length and serve as a method of predicting cycle length.

It is known that ovulation occurs during the nadir of progesterone production however, pinpointing ovulation by progesterone analysis seems unlikely. Timing of ovulation during this stage of low progesterone concentration may provide some answers to the repeat breeder cow problem often observed. It is possible these repeat breeder

Fig. 8
UBC "Normal" Cycles
Combined

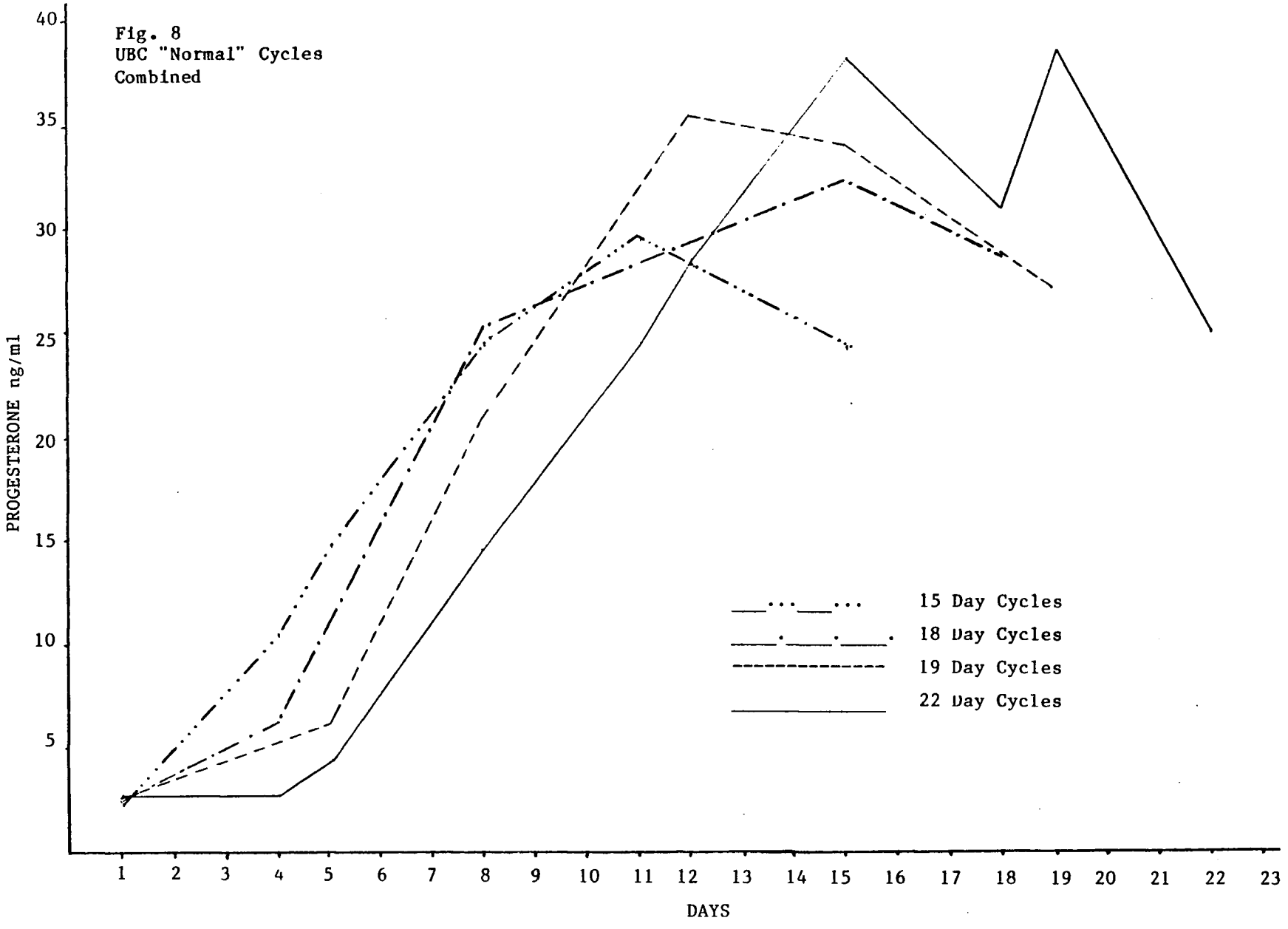
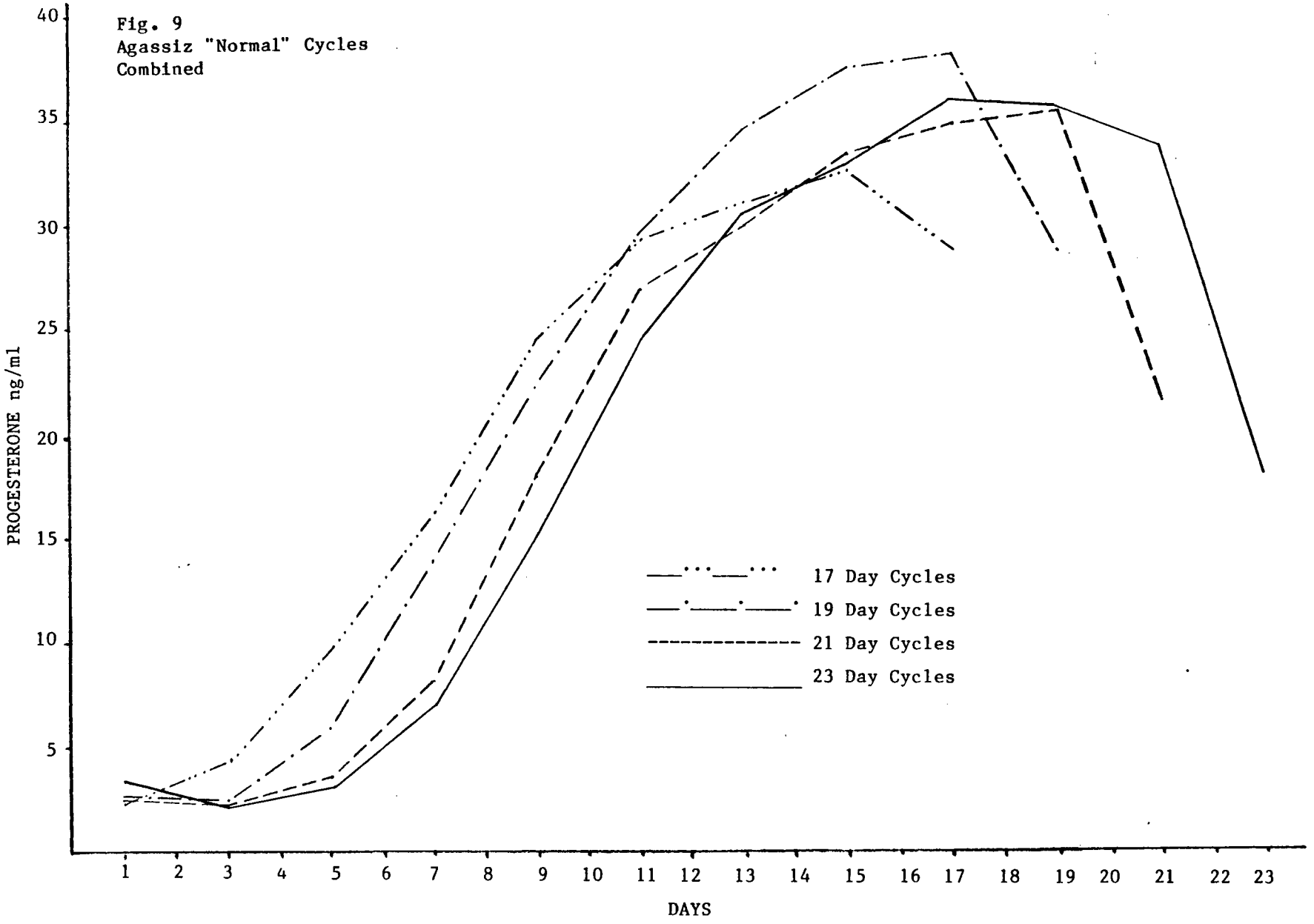


Fig. 9
Agassiz "Normal" Cycles
Combined



cows remain in phase one longer than those cows which conceive readily making timing of insemination difficult. This stage of the reproductive cycle deserves further examination.

When the various profiles for the "normal" reproductive cycle were superimposed one upon another it was found that the slopes for the rise in progesterone concentration from approximately 4 ng/ml milk to the peak did not differ markedly between any of the cycle lengths nor between the UBC data and the Agassiz data.

Longer cycles did tend to remain longer at a greater concentration of progesterone.

4. Progesterone Profiles

Many graphs of the cycles were produced to facilitate monitoring the reproductive status of the animals being sampled. As soon as possible the results of the radioimmunoassay were forwarded to both farms, so that the herdsman would have a better understanding of the status of the animals, and could more accurately predict upcoming estrus periods.

As has been noted, some animals not having been observed in estrus were mated on the basis of previously predicted ovulations and cycle lengths. Many of these animals conceived, unfortunately accurate records of this aspect were not maintained and therefore proof cannot be offered.

A great variety of types of cycles were noted and examples of these follow.

Animals exhibiting a delayed start to ovarian cycles such as cow 73025 Fig. 3 are easily missed as most dairymen do not concern themselves with the reproduction of the dairy cow until approximately 50 to 60 days post-partum when they feel that the animal should be ready to breed to maintain a yearly calving interval. They may be included with any cows to be palpated at this time if records of early detected heats are kept and it is noted that they have not had a heat recorded, or if some other abnormality is noted. Palpation of the ovaries is an extremely delicate and somewhat subjective technique. Through the use of the progesterone profiles we can identify these problem animals and in conjunction with palpation, treatment can be undertaken to shorten the period of ovarian inactivity.

Short first cycles are noted in the graphs for cows 72007, Fig. 10; 73017, Fig. 11; 73021, Fig. 12, and is speculated from the graph for 74004, Fig. 13. The short first cycle shown in Fig. 11; 73017 may be due to treatment stimulating the reproductive tract.

The graph for cow 71008, Fig. 2, illustrates a normal first cycle and second cycle followed by a successful breeding. This animal shows the ideal reproductive performance with a calving interval well under 365 days.

The graph for cow 72007, Fig. 10, shows a short first cycle followed by the development of a corpus luteum cyst on the right ovary. This graph also shows the immediate response to treatment of prostaglandin with a return to cycling behaviour.

Treatment for reproductive problems is not always successful. This is illustrated by the graph for cow 73021, Fig. 12. This graph

shows a normal cycle followed by the growth of follicular cysts on both the right and left ovaries. These did not respond to manual expression. Estrus was detected two days after treatment with ECP® (estradiol cypionate) and was associated with an unsuccessful breeding. This was followed 40 days later with an ineffective treatment of prostaglandin following palpation which detected an inactive left ovary and a cystic right ovary. This animal continued to exhibit signs of nymphomania and was subsequently slaughtered.

The graph of cows 74004 Fig. 13 and 73017, Fig. 11 show possible early embryonic abortion. Cow 74004 Fig. 13 illustrates a breeding followed by high progesterone values for a period of about 40 days followed by a precipitous drop and subsequent estrus. Cow 73017, Fig. 11, shows high values following breeding for 30 days and a subsequent drop in value.

A short quiescence is illustrated in graphs for cows 71008, Fig. 2; 72007, Fig. 10; and 74016, Fig. 14. Abnormally long cycles are illustrated in the graph of cow 74016, Fig. 14. This animal exhibits an abnormally long cycle length of 28 days illustrating the variability between animals.

5. Pregnancy Diagnosis

Keeping in mind the constraints of the analysis as stated by Heap et al. (1973) and Heap et al. (1976) a progesterone concentration indicative of the luteal stage or Phase 3 of the reproductive cycle, 12 ng/ml milk, was used as a cut-off value for the pregnancy test.

Fig. 10
Cow No. 72007

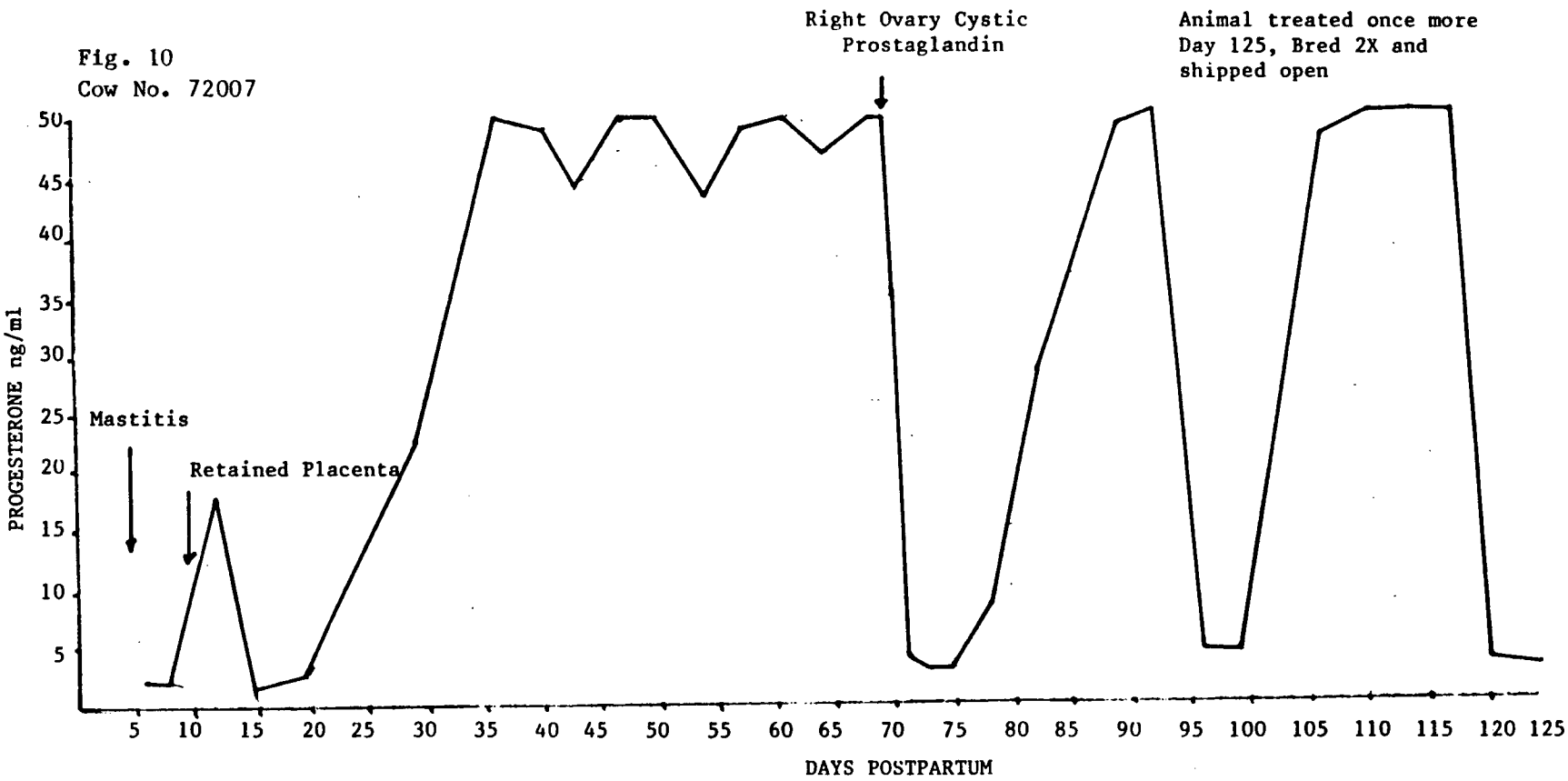


Fig. 11
Cow No. 73017

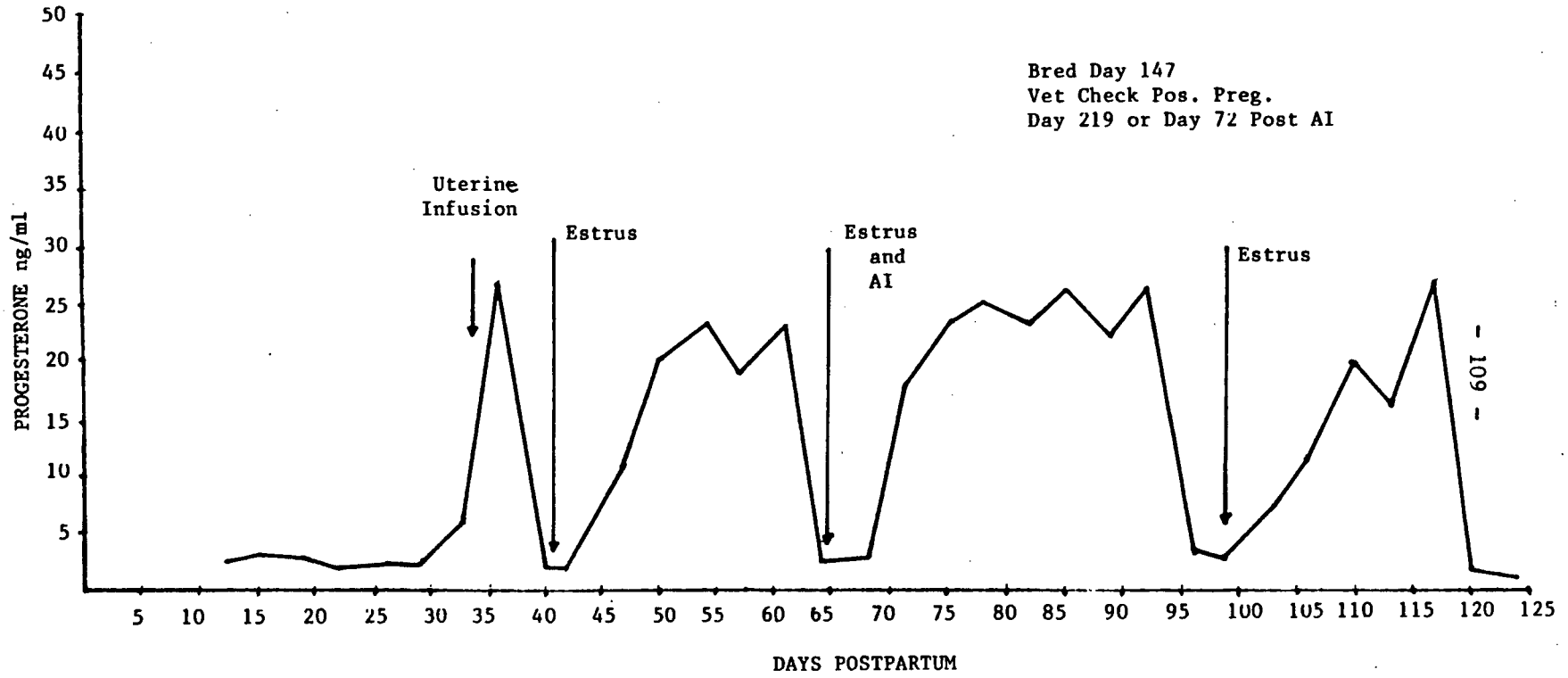


Fig. 12
Cow No. 73021

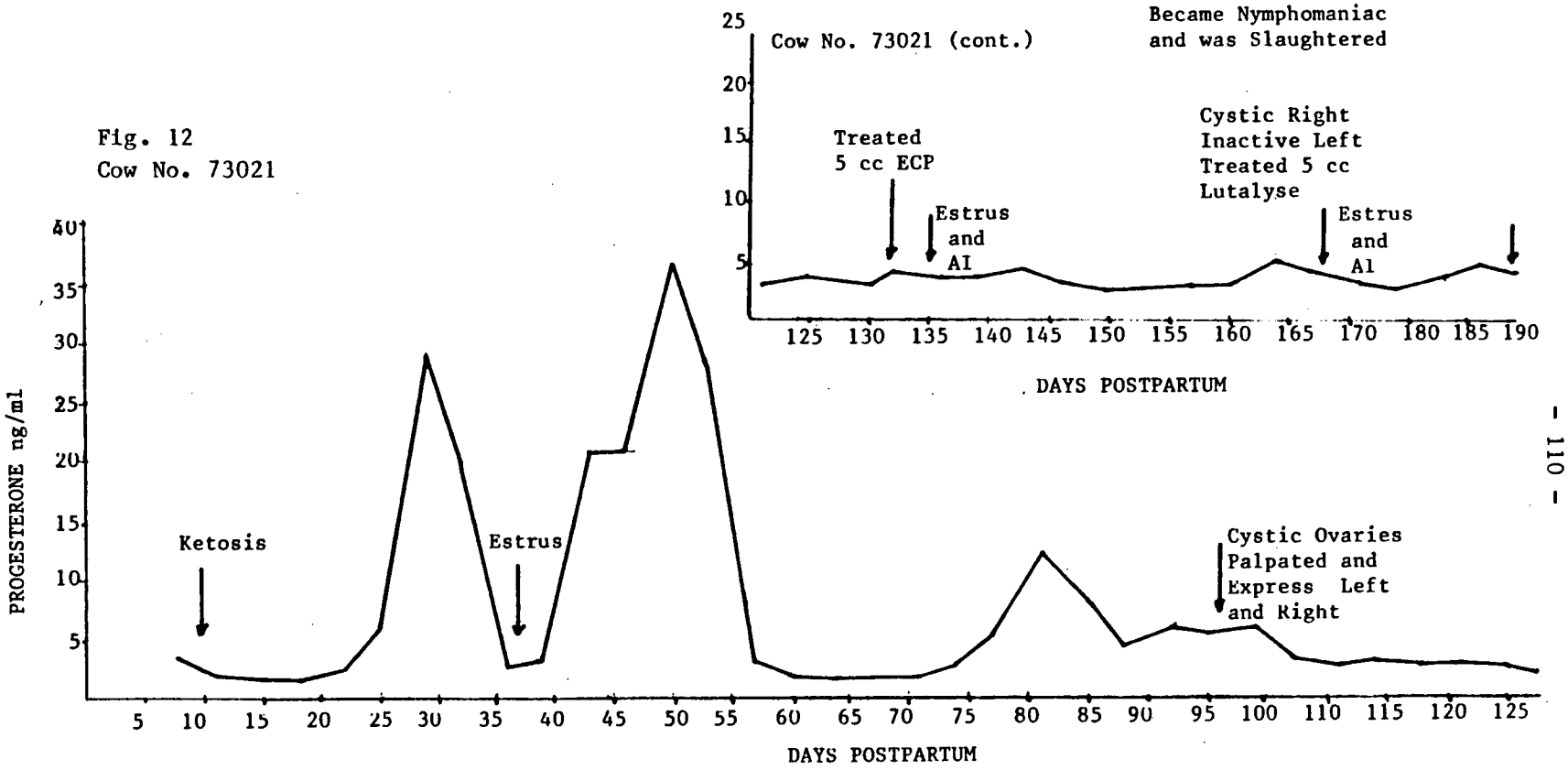


Fig. 13
Cow No. 74004

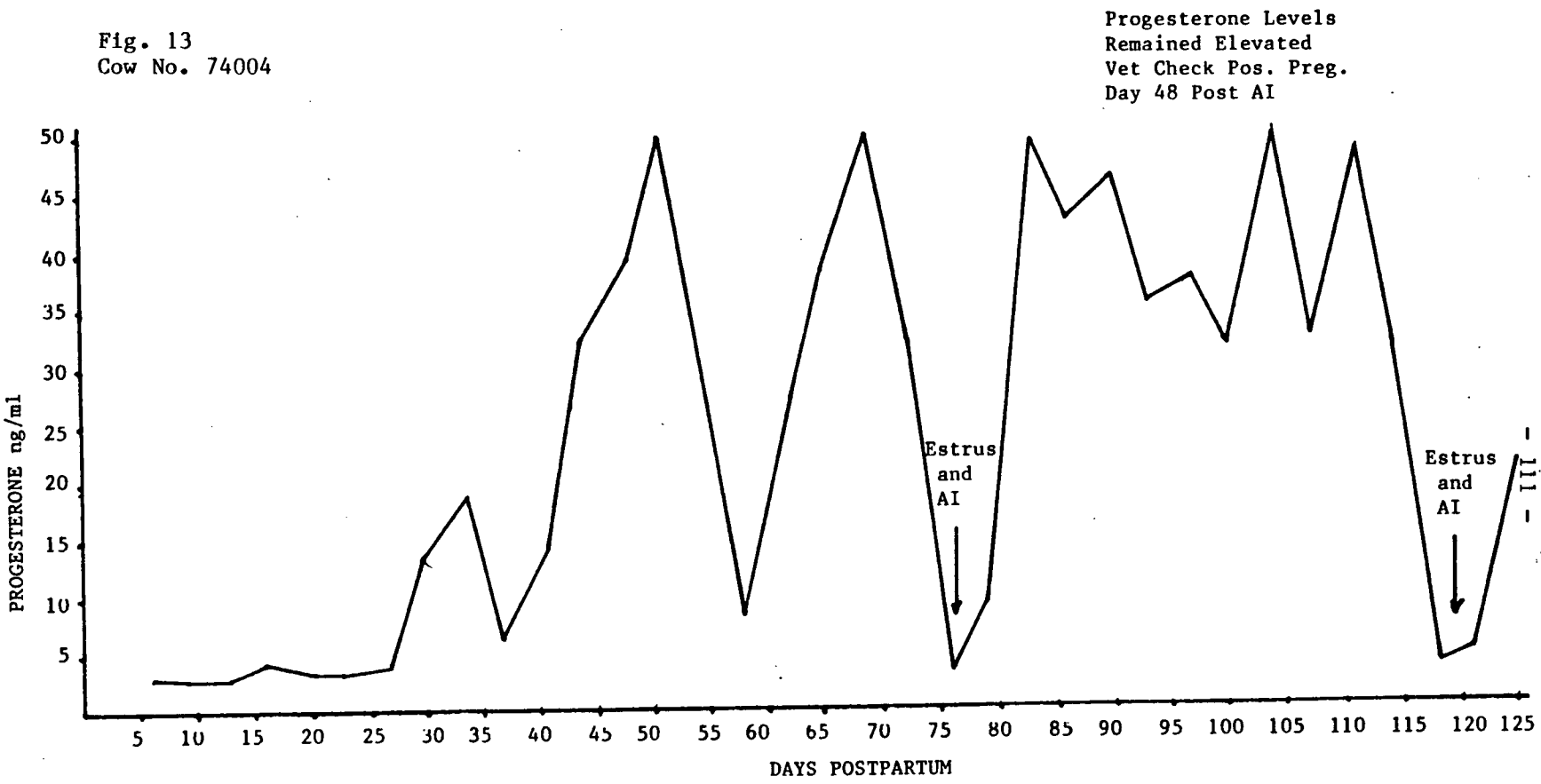
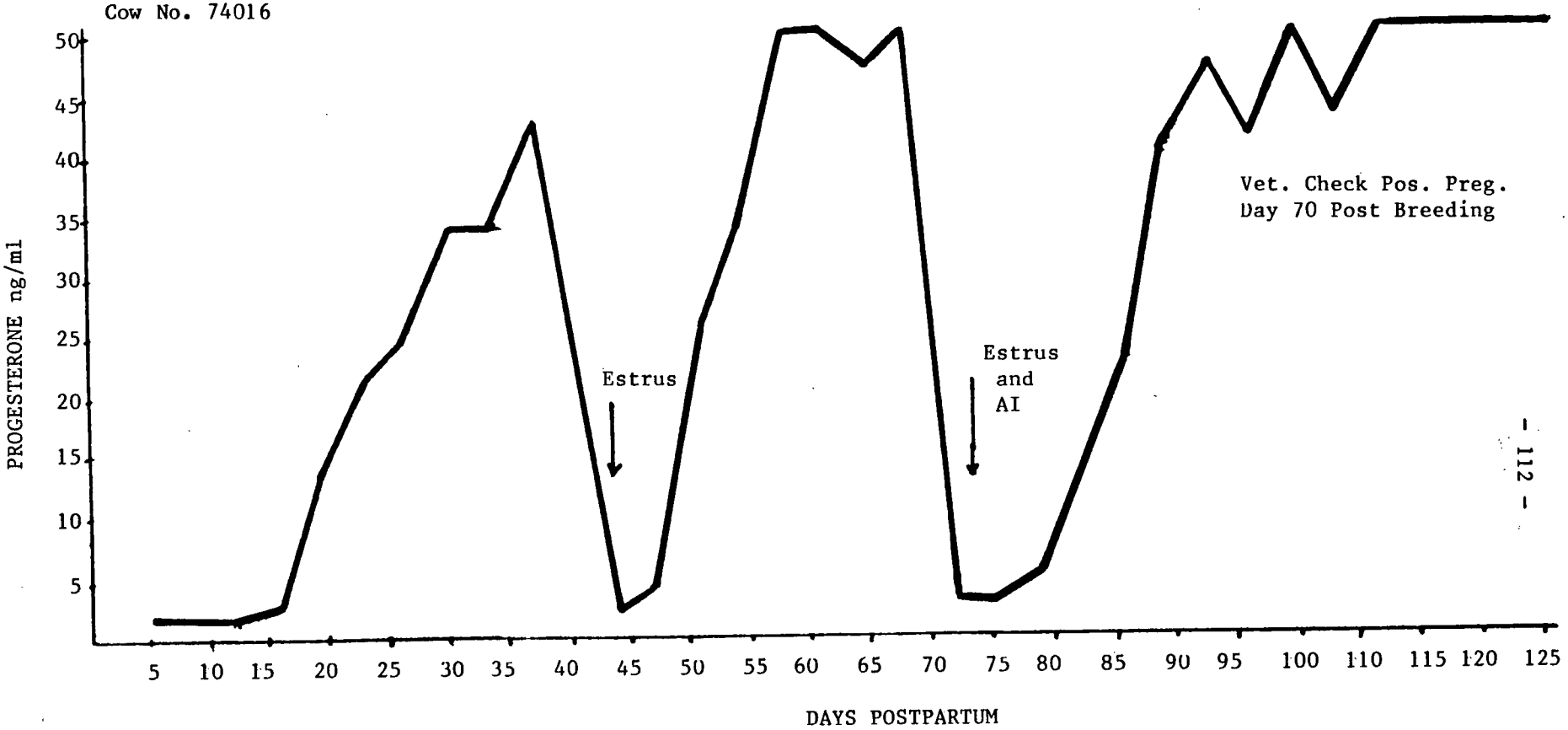


Fig. 14
Cow No. 74016



Those values above 12.0 ng progesterone per ml milk signified a pregnancy while those below were diagnosed open.

5.1 UBC Data

The results of the progesterone pregnancy test for UBC data are given in Table 22.

There was a total of 28 day 21 values from the UBC data of which 64.3% or 18 animals were diagnosed pregnant. Of these, three animals were incorrectly diagnosed pregnant, for a success rate of 83.4% for positive diagnosis. Of the 10 animals diagnosed open, all were correct.

If the value at breeding is considered, two of the three cases diagnosed incorrectly positive would have been rejected as having been bred at the wrong time. With a two sample test, that is a sample on day of insemination as well as a sample on day 21, the success rate for positive diagnosis rises to 93.8%. Also, one of the cases diagnosed open had a value at breeding of 11.5 ng progesterone per ml which would have served as an early warning of an incorrectly timed breeding.

For those tested for pregnancy with a sample collected at 22 days post-breeding, the test proved once again 100% correct in predicting six cases open. The pregnant diagnosis was again correct in 83.4% of 18 cases. Incorrect timing of insemination would have eliminated one of the three incorrectly diagnosed pregnant cases giving a two sample test success rate of 88.2% for positive diagnosis.

There were 13 values for day 23, nine of which were correctly diagnosed pregnant and four were correctly diagnosed open. The day 24

Table 22

Pregnancy Test Results (UBC Data)

	Positive Correct		Negative Correct		Positive Incorrect	
	Value at Breeding	Value at Test Day	Value at Breeding	Value at Test Day	Value at Breeding	Value at Test Day
Day 21	(15) 2.76 ± .69	(15) 43.06 ± 8.40	(10) 3.79 ± 2.68 (9)* 2.93 ± 0.80	(10) 3.57 ± 1.33	(3)+ 16.96 ± 17.74	(3) 37.66 ± 8.81
Day 22	(15) 2.56 ± .61	(15) 39.67 ± 10.30	(6) 10.36 ± 17.63 (5)* 2.44 ± .67	(6) 3.06 ± 1.35	(3)x 15.43 ± 17.37	(3) 26.33 ± 6.85
Day 23	(9) 2.81 ± .76	(9) 40.10 ± 14.04	(4) 2.35 ± .56	(4) 2.35 ± .49		
Day 24	(4) 3.37 ± 1.78	(4) 49.25 ± 1.29	(1) 4.5	(1) 2.1	(1) 2.6	(1) 22.0

Note: Number of observation in brackets ( ).
Value given is mean ng progesterone/ml post milk stripping ± S.D.
*One sample with a high value omitted
+One sample value 42.0 ng/ml included.
x One sample value 40.0 ng/ml included.

test proved 80% accurate in diagnosing correctly four of five cases pregnant and one case was correctly diagnosed open.

Combining all the values for the UBC data we find that 50 cases were diagnosed pregnant with an accuracy of 86.0%. A two sample test increases this accuracy to 91.5%. Although there were a total of seven incorrect positive diagnosis of which only three can be explained by improper timing of insemination, further examination of the UBC data shows that three animals also fit the criteria set down for cycle Type 5 indicative of early embryonic abortion. With the criteria for cycle Type 5 requiring the concentration to remain greater than 12 ng progesterone per ml. milk, the remaining incorrect positive diagnosis would have remained high longer than normal yet not long enough to fit into the Type 5 category.

5.2 Agassiz Data

The results of the progesterone pregnancy test for the Agassiz data are given in Table 23.

As the Agassiz herd was sampled every other day, pregnancy testing was done for days 21 and 22 post-insemination. From 75, day 21 values, 70.7% or 53 cases were diagnosed pregnant with a success rate of 90.56%. There was an accuracy of 100% in the diagnosis of 22 cases open.

From 74 day 22 values, 70.3% or 52 cases were diagnosed pregnant with a success rate of 90.38%. The diagnosis of open again proved 100% accurate in 22 cases.

Table 23

Pregnancy Test Results (Agassiz Data)

	Positive Correct		Negative Correct		Positive Incorrect	
	Value at Breeding	Value at Test Day	Value at Breeding	Value at Test Day	Value at Breeding	Value at Test Day
Day 21	(48) 1.95 ± .52	(48) 38.97 ± 10.85	(22) 2.14 ± 1.11 (21)* 1.92 ± 0.5	(22) 2.98 ± 1.89	(5) 2.66 ± 1.24	(5) 29.76 ± 14.37
Day 22	(47) 2.29 ± .59	(47) 35.87 ± 10.95	(22) 2.51 ± 1.46 (20) 2.08 ± 0.59	(22) 2.95 ± 2.08 (21) 2.55 ± 1.03**	(5) 13.7 ± 14.48	(5) 31.4 ± 10.27

Note: Number of observation in brackets ( ).

Value given is mean ng progesterone /ml post milk strippings.

*One high value omitted.

**One sample near the cutoff value of 12.0 ng/ml omitted.

Of the total 10 cases of incorrect pregnant diagnosis in the Agassiz herd, 30% or three animals were bred at an obviously incorrect time. With a two sample test and the removal of these values, the accuracy of the pregnant diagnosis for the combined day 21 and day 22 values rises to 93.1%. Further examination of the Agassiz data shows that four animals also fit the criteria set down for cycle Type 5, indicative of early embryonic abortion.

5.3 Pregnancy Diagnosis Discussion

These results closely correspond to those found by Booth and Holdsworth (1976) who reported on a commercial service with non-pregnant test results close to 100% and an error rate of approximately 10% in pregnant test results due mainly they felt to early fetal death, to those reported by Eastman (1979) who reported an accuracy rate for pregnancy of 83.3% for 30 cows, and to those reported by Foote et al. (1979) who reported an accuracy rate of 98% for 126 non-pregnant animals and 80% for 180 predicted positive.

Bulman and Lamming (1977) through the use of sequential sampling of the progesterone concentration in milk, showed that prolonged luteal activity could occur in the cow in the absence of any apparent clinical abnormality. It is, therefore, possible that some of the remaining unaccounted for false pregnancies could be attributed to these animals experiencing persistent luteal activity without being pregnant.

The radioimmunoassay of progesterone in milk provides a very reliable means of detecting non-pregnancy and a reasonably accurate

means of detecting pregnancy with a single sample taken on day 21 to day 24 post-insemination. The addition of a sample on day of breeding increases the accuracy of the pregnant diagnosis to greater than 90%. This could provide the producer the ability to rebreed the non-pregnant cow at an earlier date reducing the cost of production associated with increased days open. Those animals incorrectly diagnosed pregnant as well as those animals correctly diagnosed pregnant which subsequently experienced early embryonic abortion may be found if a rectal palpation for pregnancy is performed at approximately 60 days post-insemination.

6. Estrus Detection And Reproductive Performance

Combining both UBC and Agassiz data for an assessment of estrus detection and assigning a value of less than 5 ng progesterone per ml post-milk strippings as a correct value for estrus, from a total of 220 cases only nine cases or 4.1% were incorrectly detected in estrus.

McCaughey and Cooper (1980), studied 1177 milk samples taken from cows being inseminated and found 91 animals or 7.7% had levels of milk progesterone in excess of those observed at normal estrus.

Separating the two herds we find near average accuracy in heat detection with the UBC data showing six incorrect timing out of 71 cases for 8.5% incorrect timing of insemination. It must be noted that one of the incorrect timing of insemination cases did result in a pregnancy. This lowers the true incorrect timing of insemination to five from 71 cases, or 7.0%.

The Agassiz data shows remarkable accuracy in timing of insemination with only three of 149 cases or 2.0% being performed at a

time when the milk sample concentration of progesterone was greater than 5 ng/ml.

The number of days from parturition until the first detected estrus, or in the case of an animal with no early estruses noted, until the first breeding, was compared to the number of days from parturition until the first progesterone predicted estrus. Values are listed in Table 6.

Days to first progesterone detected estrus was determined as the number of days from parturition until a rise in progesterone concentration of greater than 5 ng/ml milk and back to less than 4 ng/ml milk.

The mean, standard deviation, and range, in days post-partum for first visually detected estrus for 42 animals in the UBC herd was 49.79 ± 21.67 days, with a range of 90 days. This compares to 41.0 ± 15.14 days, and a range of 73 days, for first progesterone predicted estrus for 40 animals. There are less animals in the progesterone predicted estrus group due to the fact that some animals lacked early sampling.

A greater difference was noted in the Agassiz data when comparing these tests. The mean, standard deviation, and range, in days post-partum for first visually detected estrus for 112 animals was 53.02 ± 29.14 days, with a range 175 days, compared to 34.34 ± 11.67 days with a range of 66 days, for first progesterone predicted estrus for 101 animals.

Nearly one full heat, or 18.68 days for every animal was being missed in the Agassiz herd when these two values were compared. Looking

at the range in values, we find the possibility that one animal was cycling for up to (175-66) 109 days without being noticed.

The mean, standard deviation, and range, in days to first service, for 42 animals in the UBC herd was 74.86 ± 22.21 days, with a range of 87 days, compared to 82 ± 23.94 days, with a range of 152 days for 112 animals in the Agassiz herd. The greater number of days for the Agassiz herd may reflect the imposed breeding regime.

Further reproductive performance data is given in Table 24. When viewing reproductive performance results for both these herds, the fact that both herds are used for various experimental procedures must be taken into account. For example, during the period of sampling for this data base, the Agassiz herd was also being used for another study involving early and late breeding regimes. As well the herd was being drastically reduced in number. With respect to the UBC herd, normal culling and breeding times were employed in conjunction with some feeding trials. These factors must be considered in judging the results of any reproductive performance tests. Only animals with the most complete data available were used in arriving at these values.

Comparing the results listed in Table 24 with those of De Kruif (1978), as mentioned in the literature review, we find that the mean number of days open for the UBC herd of 96.48 days comes close to De Kruif's (1978) ideal of an average interval of 85 days between parturition and conception. This allows for an average calving interval for the UBC data of slightly over a year. Four animals in the UBC herd had days open ranging from 165 - 184 days giving them calving intervals

Table 24

Reproductive Performance

Indicator	Agassiz		UBC	
	Mean Value ± Std. Dev. (Range)	No. Cases	Mean Value ± Std. Dev. (Range)	No. Cases
Days Open	111.75 ± 47.83 (246)	112	96.48 ± 34.95 (136)	42
Days to First Service	82.32 ± 23.94 (152)	112	74.86 ± 22/21 (87)	42
Days to first Progesterone Detected Estrus	34.34 ± 11.67 (66)	101	41.0 ± 15.14 (73)	40
Days to First Visually Detected Estrus (or First Breeding)	53.02 ± 29.14 (175)	112	49.79 ± 21.67 (90)	42
Days from Conception to Positive Vet Check	52.01 ± 13.19 (74)	97	60.43 ± 23.59 (96)	42

of nearly 15 months. These animals would have to exhibit great persistency in this lactation or extremely high production to warrant keeping them for the predictably long dry period of 140-159 days, if they were milked for only the regular 305 day production record.

Days open for the Agassiz herd was greater at a mean level of 111.75 days. This would be reflected in a calving interval of close to 13 months. Again this may reflect the restraint of a specified breeding regime enforced upon this herd.

Similar to the UBC herd 19 animals in the Agassiz herd show number of days open greater than 150 days, and even as high as 277 days. Days open should be included in the criteria for culling for the most efficient and economical management strategy.

Neither the UBC herd, nor the Agassiz herd, compare well with De Kruif's (1978) ideal of 80% conception after the first insemination. Only 25 of the 42 animals in the UBC data, to have a positive veterinary check for pregnancy, conceived to a first breeding. This represents only 59.5% pregnant after first service. One additional animal conceived to a double breeding with first and second breedings only one day apart. Including this animal only increases the percent pregnant after first service to 61.9%.

With respect to the Agassiz herd, of the 97 animals to receive a positive veterinary check for pregnancy, only 48 animals or 49.48% conceived to first service. If an additional seven animals who were bred a second time within six days of first service, and conceived, are included, this brings the pregnancy rate to first service to only

56.70%. These multiple services, within six days, may be explained by a controlled breeding system following hormonal treatment, or possibly inaccurate initial heat detection.

Regarding the number of services per conception, De Kruif in 1978 stated that an ideal number to strive for would be 1.3 services per conception. In this respect, both the UBC, and the Agassiz herds, required more services per conception but were similar with values of 1.79 services per conception on 42 conceptions and 1.88 services per conception on 97 conceptions, respectively.

Both of these herds showed examples of cows being bred up to eight times, and are reflected in those animals with extended number of days open.

The mean, standard deviation, and range, in days from conception until a positive palpation was 60.43 ± 23.59 days, with a range of 96 days for 42 animals in the UBC herd. The Agassiz herd received confirmation of pregnancy by veterinary palpation earlier, 52.01 ± 13.91 days, with a range of 74 days.

SUMMARY AND CONCLUSIONS

The length of time spent in quiescence post-partum can greatly affect reproductive parameters such as days to first estrus, which in turn may affect days to first breeding and ultimately calving interval. Progesterone analysis and the interpretation of its concentration over time provides an effective non-invasive technique to monitor this important aspect of fertility.

By establishing a normal period of quiescence for a herd the use of progesterone radioimmunoassay of sequential milk samples may provide the researcher with a means of assessing the effects of various treatments and diets on reproductive activity, without the invasive technique of rectal palpation which has been shown to affect the reproductive tract.

Because two different herds have been sampled under two different sampling regimes no definite conclusion can be drawn from the comparisons of the results. Differences noted between the two data sets may be due to sampling technique or possibly to factors associated with the particular herd.

For a comparison of sampling regimes, one herd should be sampled daily and from this data set different sampling regimes can be compared.

The classification of the cycle as described did not adequately cover the fourth phase of the estrus cycle when the corpus luteum regresses as a new follicle matures, and led to the standard curves for the "normal" 22 and 23 day cycles not necessarily beginning at the nadir of progesterone concentration.

The combination of the various "normal" cycles showed that much of the increase in cycle length occurs during the first phase of the cycle when the concentration of progesterone was less than 4 ng/ml milk. As the cycles lengthen out, the time spent in phase one increases.

The slopes for the rise in progesterone concentration over time for the "normal" cycles did not differ markedly between any of the cycle lengths nor between the UBC data and the Agassiz data.

The progesterone profiles produced for each of the animals provided the herdsman with a means of predicting estrus, monitoring reproductive treatments and reducing the number of animals that must be observed for estrus through the early identification of pregnant animals.

Due to the number of incorrect positive pregnancy determination and the number of these which could be identified as having been bred at an incorrect time, it would be this researchers recommendation that when progesterone radioimmunoassay is used in a pregnancy determination role a sample be taken at breeding to be compared with the sample taken on day 21.

Examination of the profiles of progesterone concentration vs. time of all the animals sampled shows that sampling twice per week was adequate for determining abnormal cycles. The increased number of short first cycle classifications found in the Agassiz data compared to the UBC data shows that accuracy in determination of reproductive status increases with the frequency of sampling. The limited number of

classifications identified in Phase 4 suggests that daily sampling at least in this phase of the cycle may be required to accurately monitor the regressing corpus luteum stage.

It is therefore this researchers opinion that the radioimmunoassay of progesterone from milk samples collected in a sequential manner and the reproductive profiles derived from them, may provide the producer a useful means of meeting the needs of improved reproductive efficiency as stated by Melrose (1979).

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