THE UNIVERSITY OF MANITOBA

MILK PROGESTERONE AS AN INDEX OF OVARIAN ACTIVITY IN THE POSTPARTUM DAIRY COW

by

Francis Ransford Tekpetey

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ABSTRACT

Milk and blood samples were collected concurrently from cows during a normal estrous cycle to determine the relationship between milk and peripheral blood levels of progesterone. A non-extraction radioimmunoassay (RIA) procedure was used to determine the concentration of progesterone in the milk. The same procedure was subsequently used to determine the concentration of progesterone in milk samples collected on days 1, 2, 3, 10, 17 and daily thereafter until day 60 postpartum from 52 heifers and cows which calved between May, 1978 and April, 1979. The progesterone profiles thus obtained were used to evaluate postpartum ovarian activity.

The pattern of change in mean progesterone levels during the estrous cycle was similar in milk and blood serum with a highly significant correlation ($r = .91$, $p < .01$) between levels in the two fluids. The levels of progesterone in the two fluids were comparable around the time of estrus, but milk levels were generally 3 to 4 times higher than serum levels at other stages of the cycle.

The milk progesterone profiles indicated that all animals had resumed ovarian activity by day 17 postpartum. Many of the animals (31 of 52 or 59.6%) did not appear to exhibit any abnormal ovarian activity prior to the first

postpartum ovulation. In these animals, milk progesterone generally remained lower than 3 ng/ml after calving; but a transient rise occurred about 4 to 7 days prior to the first ovulation. Seventeen other animals had high progesterone levels (> 40 ng/ml in some cases) after calving for a variable period of time, but not beyond day 30 postpartum. The average length of the first postpartum cycle was estimated to be 20 days which is comparable to that of a normal cycle. Out of a total of 41 animals for which post-calving breeding data were available, 46.3% (19 of 41) exhibited variable degrees and types of abnormal ovarian activity during the postpartum period as evidenced by: (i) high progesterone levels with no apparent ovulation until after day 50 to 60 postpartum which may be an indication of an incidence of follicular cysts or luteinization of developing follicles (3); (ii) extended first postpartum cycle lengths ranging from 26 to 39 days (9); (iii) failure of corpus luteum regression after the first ovulation, indicated by persistent high progesterone levels after that ovulation (6); (iv) hypo-ovarian activity indicated by low progesterone levels throughout the 60-day postpartum period (1). The average interval from calving to subsequent conception was non-significantly ($P > .05$) longer for these animals (141 ± 50 days, mean $\pm$ SE) compared to those showing no abnormalities (114 ± 36). Number of inseminations per conception was not affected.

Average interval in days from parturition to first ovulation was significantly ($P < .05$) longer for animals aged 2 years at the time of calving (27.4 ± 1.7) than for those aged 3 to 4 years (24 ± 1.2) and 5 years or more (24 ± 1.7). Animals which calved in the fall and winter (Sept. 21 - March 21) had a significantly ($P < .05$) longer interval to first ovulation (27.6 ± 1.2) than those which calved in spring and summer (22.6 ± 1.1). Level of milk production did not affect the interval to first ovulation but appeared to compound the seasonal effect since the high milk producers which calved in fall and winter had a significantly ($P < .01$) longer interval to first ovulation than their counterparts which calved in spring and summer.

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INTRODUCTION

Profitable dairying requires high milk production maintained by efficient herd management. Reproductive performance constitutes one significant aspect of herd management that requires careful attention. Reproductive activity during the postpartum period of the dairy cow influences post-calving breeding efficiency, interval to successful conception, calving interval, and subsequently milk production. Research indicates that maximum lifetime production of dairy cattle can be achieved with an optimum calving interval of 11 to 12 months. With current management practices, this would require shortening the interval to first insemination postpartum to about 50 to 60 days in order for cows to conceive by approximately 85 days postpartum.

With the necessity of a short interval to first insemination, the detection of reproductive activity during the early postpartum period becomes vital to maintaining high reproductive efficiency. One of the current techniques used to gain a better understanding of all phases of reproductive function is the determination, by radioimmunoassay (RIA), of circulating levels of various reproductive hormones that control the activity of reproductive organs.

Peripheral plasma progesterone concentration, for example, has been shown to be a useful monitor of corpus luteum (CL) function during all phases of reproductive function (Thibier et.al., 1973). Over the past ten years, progesterone has been shown to be present in milk, and changes in its concentration during various stages of the reproductive cycle parallel those seen in peripheral plasma (Darling et.al., 1972; Heap et.al., 1973; Hoffmann and Hamburger, 1973; Thibier, 1974). The ease of milk sampling coupled with the establishment of a rapid RIA procedure for the determination of progesterone in milk which does not require a solvent extraction of the steroid fraction, has given milk a considerable advantage over plasma as a biological fluid for assay. The measurement of milk progesterone at various phases of reproduction has therefore been developed as a routine practical aid in pregnancy diagnosis and estrus verification in countries such as Britain, Germany, France, the United States, and more recently Canada. While research continues in these areas, studies involving the use of milk progesterone to monitor ovarian activity during the early postpartum period are limited. Routine measurement of milk progesterone during this period could help predict the time to start postpartum insemination prior to 60 days or aid in the early identification and correction of minor transient ovarian abnormalities that may otherwise extend the postpartum anestrous period. This study was initiated, therefore, with the following objectives in mind:

(1) to monitor postpartum ovarian activity of dairy cows using milk progesterone determination,

(2) to identify and perhaps determine the effect of abnormal ovarian function in the early postpartum period on the subsequent interval to successful conception, and

(3) to determine the effect of age, season of calving and early postpartum milk production on the interval to first postpartum ovulation as indicated by the milk progesterone profile.

LITERATURE REVIEW

This review is divided into three major parts. The first part provides a brief summary of recent research data regarding progesterone levels in serum and milk and their pattern of change, which reflects luteal activity during the estrous cycle. The next section will deal with various aspects of postpartum reproductive activity in cattle. The nature of these activities and the progesterone profiles associated with them will be discussed. Postpartum estrus and ovulation, breeding and conception, calving intervals, and factors that affect these events will also be presented. The final part will focus on factors that influence the level of progesterone in milk and the possible use of milk progesterone information in reproductive management.

Progesterone Changes During the Estrous Cycle

Reproductive hormones known to be involved in the mechanisms regulating the bovine estrous cycle include; luteinizing hormone (LH), follicle stimulating hormone (FSH), estrogen and progesterone. Progesterone, a key hormone in regulating the estrous cycle, is secreted mainly by the corpus luteum. Therefore, changes in circulating levels of progesterone reflect changes in the functional status of the CL as well as the reproductive status of the animal in general.

Different assay techniques used to study progesterone levels in both peripheral blood and milk have provided information on progesterone changes during the entire estrous cycle (Stabenfeldt et.al., 1969; Pope et.al., 1969; Hendricks et.al., 1970; Snook et.al., 1971; Garverick et.al., 1971; Robertson, 1972; Heap et.al., 1973; Gartland et.al., 1975). Although there is good agreement among various workers regarding the general pattern of change, absolute progesterone levels at different stages of the estrous cycle vary considerably. Progesterone levels are low at estrus and for a few days thereafter. A rise begins on day 3 or 4 after estrus and a peak is reached at about day 11 of the cycle. Levels fluctuate slightly over the next few days, until about 48 hours prior to the next estrus; a precipitous fall to the typical estrous levels then occurs. In both dairy and beef cows, blood progesterone varies from undetectable levels to about 1.5 ng/ml at estrus and remains below 3 ng/ml until about 3 to 4 days after estrus (Hendricks et.al., 1970, Snook et.al., 1971; Robertson, 1972; Christensen et.al., 1974; Appleyard and Cook, 1976). However, a much higher value of 5.1 ng/ml for the day of estrus and 4 days thereafter was reported by Garverick et.al., (1971). The average mid-cycle peak values of blood progesterone range from a low of 3 ng/ml (Robertson, 1972) to a high of 12.6 ng/ml (Garverick et.al., 1971) and occur between day 11 and 18 of the cycle. However, most workers have reported average values of approximately 6 ng/ml for the same period.

Milk progesterone levels have been shown to parallel those reported for peripheral blood during the estrous cycle, although they may be much higher (Heap et.al., 1973; Schiavo et.al., 1975; Mauer et.al., 1974; Matuszezak et.al., 1974; Gartland et.al., 1975; Dobson et.al., 1975). Most reports indicate that at the time of estrus, milk progesterone values range from 1 to 3 ng/ml. However, lower values (0.5 ng/ml or less) comparable to the values frequently reported for peripheral blood at estrus, have also been reported (Hoffmann et.al., 1976; Pennington et.al., 1976). Similarly, although most reports indicate that the mid-cycle peak values average approximately 10 ng/ml, higher values averaging 20 ng/ml (Pennington et.al., 1976a) and above 30 ng/ml (Hoffman et.al., 1976) have been reported for the period from days 11 to 18 of the cycle.

The differences in relative progesterone concentration at a particular stage of the cycle reported by different authors, may reflect individual animal differences, as well as differences in assay techniques employed. In addition, the estimated concentration of milk progesterone will depend on whether the assay was performed on whole milk, skim or cream samples, since milk progesterone concentration correlates highly with milk fat concentration (Hoffman and Hamburger, 1973; Nuti et.al., 1975; Pennington et.al., 1976; Pope et.al., 1976; Ginther et.al., 1976). For any one animal, however, the pattern of progesterone change over the estrous cycle provides a useful means of monitoring typical or atypical ovarian activity and cyclical behaviour.

Postpartum Reproductive Activity and Fertility

1) Ovarian activity during the early postpartum period

A number of studies have been conducted to investigate ovarian activity and the profiles of reproductive hormones during the early postpartum period of the cow (Labhsetwar et.al., 1964; Erb et.al., 1971; Darling et.al., 1974; Arije et.al., 1974; Lamming and Bulman, 1976). Wagner and Hansel (1969) and Morrow et.al., (1969a,b) have reported that in most lactating cows there is a suppression of follicular development which does not last beyond 7 to 10 days following parturition. The pituitary at this time contains more FSH than LH (Labhsetwar et.al., 1964; Saiduddin et.al., 1968a), and there is also a high and fluctuating plasma level of prolactin (Arije et.al., 1974) which might be responsible for the transient suppression of ovarian activity. The pituitary LH and FSH concentrations then increase with time, accompanied by an increase in follicle development (Saiduddin and Foote, 1964; Labhsetwar et.al., 1964; Graves et.al., 1968; Wagner et.al., 1969). Similarly, plasma levels of LH remain low until approximately 6 to 8 days postpartum when small episodic releases of LH (peaks of 1 - 2 ng/ml) begin to occur (Webb et.al., 1977). These episodic LH releases increase in magnitude for several days thereafter (Edgerton and Hafs, 1973; Kesler et.al., 1977; Fernandes et.al., 1978), and occur more frequently about 2 to 3 weeks before the first postpartum estrus (Humphrey et.al., 1976; Rawlings et.al., 1979; Stevenson and Brit 1979), with preovulatory surges of 5 to 9 ng/ml (Webb et.al., 1977) in cows which ovulated. Arije et.al. (1974) reported that follicles 8 to 10 mm. in diameter were palpable on the ovaries of beef cows 14 to 30 days after calving. These follicles grew for a few days, became atretic and were then replaced by other growing follicles.

Other hormones associated with postpartum ovarian activity have been studied. At the time of calving, the progesterone level is low and remains low for a variable period of time due to regression of the CL of pregnancy (Labhsetwar et.al., 1964; Erb et.al., 1968a,b; Oxenreider, 1968; Wagner and Hansel, 1969; Donaldson et.al., 1970; Smith et.al., 1973; Arije et.al., 1974). Peripheral progesterone concentrations greater than 1 ng/ml are associated with the presence of a CL or a luteinized follicle (Stabenfeldt et.al., 1969; Swanson et.al., 1972). Prior to the onset of the first ovarian cycle, a small rise in blood progesterone occurs, persists for 3 to 5 days and then rapidly declines (Pope et.al., 1969; Donaldson et.al., 1970; Erb et.al., 1971; Robertson, 1972; Hendricks et.al., 1972; Tribble et.al., 1973; Arije et.al., 1974; Webb et.al., 1977). Tribble et.al. (1973) observed that this transient rise in progesterone as well as 20β-dihydroprogesterone, followed a significant rise in LH and may thus be associated with follicular luteinization as suggested by Robertson (1972). Tribble et.al. (1973) also suggested that the 20β-dihydroprogesterone is required to return the system to a cyclic pattern. Similar observations of a small transient rise in milk progesterone before first ovulation have been made in many animals (Lamming and Bulman, 1976; Bulman, 1978). Estrogen concentration during the early postpartum period is highly variable. Immediately following parturition, estrogen levels (urinary and blood) drop markedly (Hunter et.al., 1970; Arije et.al., 1974) and fluctuate widely thereafter (Erb et.al., 1971; Arije et.al., 1974). Towards the latter stages of the early postpartum period (around 40 days postpartum), estrogen levels are generally several times higher than those observed during the first 2 weeks of pregnancy (Randel and

Erb, 1971) or during estrous cycles which occur 60 or more days postpartum (Garverick et.al., 1971). However, a high proportion of otherwise normal cows do not exhibit estrus during the first postpartum ovulation (Morrow et.al., 1966; Marion and Gier, 1968; Erb et.al., 1971; Robertson, 1972; Thatcher and Wilcox, 1973; Lamming and Bulman, 1976). These "silent" ovulations are normally followed by subnormal development of luteal tissue and a shortened life span of the corpus luteum. Robertson (1972) reported that a vaginal mucus discharge was generally the first indication of the resumption of ovarian activity after parturition. He explained that this mucus discharge possibly accompanies a peak release of estrogen which, in the absence of any preconditioning period by elevated progesterone levels, does not result in overt estrus but does result in a release of LH that produces either an ovulation or a luteinized follicle. Erb et.al., (1971) have postulated that;

a) the re-establishment of ovarian cyclicity after calving requires an initial overstimulation by FSH and consequent high estrogen production, and

b) high estrogen, in addition to temporarily inhibiting FSH, causes morphological alteration of the follicles and alters steroid synthesis to favour progestin production instead of estrogens, thereby initiating further release of FSH.

2) Factors that affect the length of postpartum anestrus

The literature reviewed indicates that ovarian activity is resumed fairly soon after calving (7 to 10 days). However, it seems that the endocrine balances required to support normal estrous cycles and to re-establish fertility are restored gradually. This introduces a period of postpartum anestrus in the cow, the length of which is affected by numerous factors other than disease.

Before the successful re-establishment of pregnancy following parturition, the uterus of the cow must first undergo extensive regression and regeneration of new uterine tissues, a process called uterine involution. Uterine involution occurs very rapidly after calving and normally takes about 30 days to complete (Morrow et.al., 1966; Gier and Marion, 1968; Riesen et. al., 1968; Wagner and Hansel, 1969; Rakha et.al., 1971). While the early onset of postpartum ovarian activity and ovulation is not dependent on the gross uterine involution, Marion and Gier (1968) have suggested that the characteristic short life span of the first CL after parturition may be caused by cytological conditions in the endometrium which have not yet returned to pre-gravid conditions. More recently, Foote (1978) has suggested that the major reason cows fail to exhibit normal estrous cycles following calving is poor or incomplete uterine involution. Suckling has been shown by some workers

(Lynn _et.al._, 1966; Casida _et.al._, 1968; Riesen _et.al._, 1968; Lauderdale _et.al._, 1968) to enhance uterine involution, but other investigators (Oxenreider, 1968; Wagner and Hansel, 1969; Moller, 1970; Oxenreider and Wagner, 1971; El-Fouly _et.al._, 1976) have observed little or no effect of nursing on uterine involution. The time required for complete uterine involution is generally longer in pluriparous cows than in primiparous cows (Buch _et.al._, 1955; Quevedo _et.al._, 1965; Morrow _et.al._, 1966; Marion and Gier, 1968; Araujo _et.al._, 1974) and is also longer in winter and fall than in summer and spring (Buch, 1957; Norwood, 1963; Marion _et.al._, 1968; Cheredkov, 1970; El-Fouly _et.al._, 1976; Gospodinov and Kostov, 1976).

In addition to early uterine involution, normal estrous cycles must be established early enough to ensure good postpartum fertility. There is a considerable amount of information on the interval from calving to the establishment of cyclic ovarian activity and factors that affect this interval. First ovulation normally occurs earlier in dairy cows (10 - 45 days postpartum) than in beef cows (36 - 70 days). Similarly, first estrus occurs earlier in dairy cows (30 - 72 days) than in beef cows (46 - 104 days) (Casida _et.al._, 1968; Morrow _et.al._, 1969a,b; Casida, 1971; Bulman, 1978). The longer interval to first ovulation and/or estrus in beef cows is largely attributed to suckling which has also been found to extend postpartum anestrus in dairy cows (Wiltbank and Cook, 1958; Saiduddin et.al., 1968b; Wagner and Hansel, 1969; Oxenreider and Wagner, 1971; Short et.al., 1972; England et.al., 1973; Carruthers et.al., 1977). The mechanisms by which suckling suppresses postpartum ovarian activity is not clear. However, the findings of Reisen et.al. (1968), Wagner and Hansel (1969), and Wagner (1970) indicate that it might be related to mammary stimulation which was found to affect endocrine function. Furthermore, the beef cow which is suckled several times per day compared to the twice daily milking of the dairy cow has a longer period of postpartum anestrus, even though the dairy cow loses relatively more weight during early lactation (Lamming, 1978). Ovarian activity is initiated earlier in non-suckled cows (Tribble, 1973; Connor et.al., 1974), but suckling apparently did not affect pituitary FSH concentration (Saiduddin et.al., 1968a), average serum progesterone (Tribble, 1973) or prolactin levels (Carruthers et.al., 1977). It did, however, depress average serum LH levels (Short et.al., 1972; Carruthers et.al., 1977). In addition, Webb et.al. (1977) reported that prior to 20 days postpartum, both LH and progesterone responses to intravenous gonadotropin releasing hormone (GnRH) injections were lower in suckled cows. It is generally considered that the suckling stimulus inhibits the release of gonadotropins and acts via the hypothalamus to influence the release of GnRH and prolactin inhibiting factor (PIF). In sheep, Lamming et.al. (1974) observed that the occurrence of postpartum hypo-ovarian activity was associated with hyper-prolactinemia, inferring that prolactin affects ovarian function; and several other authors have suggested that there is a direct suppressive effect of prolactin at both the ovarian (Thorner et.al., 1974) and the hypothalamic level (Thorner et.al., 1974; Aono et.al., 1976).

The level of milk production also affects the period of postpartum anestrus but to a lesser extent (Oxenreider and Wagner, 1971). The indications are that higher levels of milk production result in longer periods of postpartum anestrus (Marion and Gier, 1968; Oxenreider and Wagner, 1971). However, Olds and Seath (1953) found that while the correlation between milk production during the first 120 days after parturition and the number of days from calving to first heat was significant ($P < .05$), the level of milk production accounted for only 0.9% of the variation in the anestrous period. Lactation is an energy-demanding process. The plane of nutrition, and in particular energy intake, is therefore stated to have an important influence on reproduction during lactation (Wiltbank et.al., 1965). In a 1971 study, Oxenreider and Wagner found that both lactation and low energy intake delayed postpartum follicular growth and ovulation in primiparous Holstein females. Both factors also had a depressive effect on plasma glucose level during the first 8 weeks postpartum; plasma glucose level in turn was correlated negatively with the postpartum interval to occurrence of large follicles and subsequent ovulation. McClure (1968b) had previously demonstrated a relationship between hypoglycemia and infertility in dairy cows. With regard to how hypoglycemia may exert its influence, Howland et.al. (1966) have speculated that since brain tissue function depends on glucose for its energy source, even mild hypoglycemia may depress hypothalamic function, thus resulting in a loss of ovarian activity. It therefore seems that the level of milk production coupled with the availability of energy to the animal prior to parturition will influence ovarian activity after calving. Indeed, Carman (1955) found a very high correlation between the period from parturition to first estrus and milk production in the preceding lactation.

Somewhat related to milk production and energy intake are the effects of age and season of calving on the length of postpartum anestrus. The literature relating to the effect of age at calving is scanty and inconsistent. Both Warnick (1955) and Buch et.al. (1955) reported that age at calving appeared to have little relation to length of postpartum anestrus. On the other hand, Kohli and Suri (1957) found that the age of the cow had a great influence on the interval to first estrus which was longest for cows aged

5 to 6 years and over 12 years. The interval was similarly long for first-calf heifers; and gradually decreased to the 5th lactation. Wiltbank and Cook (1958) have also reported that there was a tendency for a shorter interval between calving and first estrus as the cow grew older. Seasonal effects on the interval to first postpartum estrus are more consistent. The interval tended to be longer when cows calved in winter compared to summer (Buch et.al., 1955: Buch, 1957; Wiltbank and Cook, 1958; Zoarol and Polasek, 1969; Moskalenko, 1970).

Associated with the first two ovulations postpartum is a high incidence of cystic follicles which may further extend the postpartum anestrus. The incidence of follicular cysts was found to increase with increased milk production (Johnson et.al., 1966; Marion and Gier, 1968), but was not related to age at calving (Marion and Gier, 1968). It has been speculated that seasonal stresses compound the effects of production stress as causes of follicular cysts, since there is a higher occurrence of cystic follicles during fall and winter (Roberts, 1955; Morrow et.al., 1966; Marion and Gier, 1968). Both Morrow et.al. (1966) and Marion and Gier (1968) have implicated cystic follicles as direct causes of delayed estrus postpartum. Most follicular cysts may, however, resolve themselves without treatment.

3) Postpartum interval to breeding

One of the important factors that influences productivity of the dairy cow is her calving interval. Extended intervals to first service and subsequent successful conception results in longer calving intervals (Bozworth, et.al., 1972; Charnetzki et.al., 1978). There is evidence to suggest that breeding early in the postpartum period can reduce the calving interval (Olds and Cooper, 1970; Whitmore et.al., 1974). According to a review by Britt (1975), long-term effects of early breeding (at first estrus or first estrus after 40 days postpartum) on reproductive performance is unclear. However, the disadvantage of early breeding appears to be lowered fertility since cows bred early require more inseminations per conception. Although service interval alone may account for 16.8% of the variance in the interval from parturition to conception (Touchberry et.al., 1959), Whitmore et.al. (1974) have reported that inseminations at first postpartum estrus did not affect fertility of inseminations later in the postpartum period. The situation can be summarized by the observations of Harrison et.al. (1974) that it was difficult to obtain consistently short calving intervals during each lactation of a cow's life in the herd.

The optimum calving interval for maximum milk production is uncertain. However, evidence indicates that increased milk production per day in the herd could be achieved by reducing the calving interval to 12 months or less (Speicher and Meadows, 1967; Louca and Legates, 1968). Through personal communications, Britt (1975) has reported that cows bred at first postpartum estrus during each of three calving intervals produced less total milk than cows bred at the first estrus after 74 days postpartum. But, since the early bred cows had a shorter average calving interval, their production per day of life was more compared to the late-bred cows. Another report referred to by Britt (1975) indicated that maximum milk yield per day between concurrent parturitions was achieved with a calving interval of 11 months. Milk yield per day decreased with either shorter or longer calving intervals. Summarizing the results of seven studies, Britt (1975) concluded that maximum milk yield per day of herd life occurred when calving interval was about 11 months. Yet, in certain individual lactations, milk yield increased as calving interval increased. High producing animals tended to yield more milk per day with calving intervals of more than 14 months compared to low producing animals which had calving intervals of less than 12 months. With proper management of the postpartum cow and adequate heat detection, it is possible to achieve a desirable calving interval of 11 to 12 months with respect to maximum milk production, by starting to breed cows at first estrus 50 days postpartum (Foote, 1978).

Milk Progesterone as a Tool in Reproductive Management

The establishment of the presence of progesterone in milk has generated considerable interest and extensive research into its potential use as a clinical tool to aid reproductive management. Among various areas of research, the use of milk progesterone level in early pregnancy diagnosis has received considerable attention (Heap, et.al., 1973; Shiavo et.al., 1975; Dobson and Fitzpatrick, 1976; Heap et.al., 1976; Hoffmann et.al., 1976; Pennington et.al., 1976; Pope et.al., 1976; Laing, 1976). Milk progesterone level is known to be closely related to the growth and secretory function of the CL in the normal cycle and in early pregnancy (Laing and Heap, 1971; Heap et.al., 1973; Heap and Laing, 1974; Darling et.al., 1974; Hoffmann et.al., 1974; Dobson et.al., 1975). Milk progesterone concentrations obtained after 20 days from the time of breeding would therefore reflect ovarian endocrine activity. Thus in non-pregnant animals, progesterone concentration after 20 days post-mating will be low since the CL regresses on about day 17 or 18 of the normal cycle; while in pregnant animals, the CL survives and hence milk progesterone level will be similar to mid-cycle levels. This forms the basis of the milk progesterone test for pregnancy diagnosis. The exact concentration of milk progesterone which forms the criteria for distinguishing between pregnant and non-pregnant animals is not consistent and is influenced by sampling technique and assay procedure. The lower limit for non-pregnancy used by various workers has varied from less than 2 ng/ml (Hoffman et.al., 1976) to less than 8 ng/ml (Pennington et.al., 1976); and the upper limit for pregnancy from greater than 7 ng/ml (Dobson and Fitzpatrick, 1976) to greater than 11 ng/ml (Pennington et.al., 1976). Similarly, the ideal time to collect milk for early pregnancy diagnosis is uncertain. Milk samples have been taken anytime between day 19 to 28 after breeding. Taking into account normal variation in cycle length, Heap et.al., (1976) have suggested that samples taken at 21 days after breeding would probably provide the most reliable results, since they were most likely to be taken during a time of low progesterone production in non-pregnant animals returning to estrus. However, Pope et.al., (1976) have proposed the following explanations that may account for false pregnancy diagnoses 20 to 21 days after breeding:

a) Ovulation may have occurred at the normal time but fertilization may have failed and the CL had a life span shorter or longer than normal. As a result progesterone concentration could have been rather higher than expected for non-pregnancy at 20 to 21 days post-breeding leading to a false diagnosis.

b) The mechanism whereby the lifespan of the CL is terminated, if no conception occurs, can fail altogether and lead to the persistence of a secretory CL.

c) There might be an abnormal delay between observed estrous behaviour when the animal was bred and ovulation; this would lead to a false pregnancy diagnosis at 21 days post-breeding.

d) Diagnosis made 20 to 21 days after breeding may be correct and subsequent findings that animals were not pregnant be due to early embryonic death.

Laing (1976) reported that experience with physical examination for pregnancy indicates that positive diagnosis made at 42 days in heifers and 50 days in cows are reliable, presumably because the period of embryonic mortality is over. Assay of samples taken at this time must however be interpreted with care since the timing does not allow any distinction between pregnancy and mid-cycle values. Irrespective of the time of sampling and the assay procedure used, there is good agreement among workers that the accuracy of negative (non-pregnant) diagnosis, as confirmed by non-return data or palpation at a later date, is higher (average of 95 - 100%) than that of positive diagnosis (75 - 80%).

Milk progesterone has also been used experimentally to plan insemination and in the diagnosis and treatment of subfertility in dairy cattle. Hoffmann et.al., (1976) measured milk progesterone at the time of insemination and found that 13% of the animals with no chance of conception could be identified at this time. This problem is associated with incorrect estrus detection due to "silent" heat. On one farm, the authors were able to correct a long series of reproductive failures by assessing the milk progesterone level at the time of insemination and drawing the farmer's attention to this failure in management. In 1976, Lamming and Bulman developed postpartum milk progesterone profiles for dairy cows and used it to identify three types of "abnormal" ovarian activity: a) hypo-ovarian activity, b) persistently high progesterone levels (persistent CL); and c) unobserved or "silent" estrus. The authors suggested the use of this method as an aid to the investigation of some types of infertility in the cow. When cows with persistent CLs and those exhibiting "silent" estrus were treated with 0.5 mg prostaglandin $F_{2\alpha}$ analogue (ICI 80996), they responded to the treatment. Treatment of cows with hypo-ovarian activity with a single intramuscular injection of 0.5 mg GnRH initiated cyclic activity as it did in an earlier study reported by Britt et.al., (1974). An alternative treatment by the insertion of a progesterone releasing intravaginal device (PRID) for 14 days resulted in subsequent luteal activity, with post-treatment estrus and pregnancy occurring in some of the animals.

In view of the considerable disagreement on the concentration of progesterone in cow's milk, researchers have long suspected that factors other than those related to assay technique are involved in determining concentration. It is well known that milk which is drawn first in the milking process contains less fat than milk drawn later, whereas there is no difference in the proportion of other major constituents of milk obtained at various times during the milking process (Smith, 1959). There is now considerable evidence to indicate that changes in milk progesterone and fat concentrations show a high positive correlation (Darling et.al., 1974; Hoffmann and Hamburger, 1973; Hoffmann et.al., 1974; Nuti et.al., 1975: Shiavo et.al., 1975: Ginther et.al., 1976). The level of milk progesterone may therefore be influenced by time and method of sampling during the milking process. This is confirmed by findings that milk from the latter stages of milking contains more progesterone (Shiavo et.al., 1975) and that evening milk generally contains more progesterone than morning milk (Darling et.al., 1974; Heap et.al., 1976). Milk progesterone concentration correlated not only with fat content but also highly with total nitrogen (Thibier _et.al._, 1976) and protein content (Heap _et.al._, 1976). The extent to which differences in milk progesterone caused by differences in milk fat level affect the assessment of ovarian activities using milk progesterone data is unclear. However, Pennington _et.al._, (1976) have indicated that the stage of the reproductive cycle was the primary determinant of progesterone concentration in milk. Cows showed greater than ten-fold changes in progesterone concentration during the estrous cycle, while milk fat percentage normally changed less than two-fold. Furthermore, they observed that although non-extracted milk yielded higher progesterone values than extracted milk, this did not render invalid the criteria used for pregnancy diagnosis.

MATERIALS AND METHODS

Experimental Animals

A total of 61 Holstein and crossbred Holstein first-calf heifers and mature cows from the University dairy herd at the Glenlea Research Centre which calved either unaided or with minimum aid between 4th May 1978 and 26th April 1979, were involved in this study. At the time of calving, the ages of these animals ranged from about 2 years to 11 years. Nine of the animals were withdrawn from the experiment for the following reasons; illness (4), death (1), violent behaviour (1), and abrupt termination of the study about two weeks earlier than scheduled because of the evacuation of the herd from Glenlea because of threatened flooding (3).

Although a research herd, the animals were fed and managed in a way that could be considered typical of a commercial operation. They were housed in individual stalls in a conventional enclosed stanchion barn. In summer and on the warm days of spring and fall, they were sent outside between 08:00 and 13:30 hours. Grain was always fed prior to both morning and afternoon milkings. Silage and/or hay was fed between milkings and at night. Water was available at all times.

Experimental Procedures

(i) Sample Collection

Each animal was put on the experiment immediately after calving. Milk samples were collected from the animal during the afternoon milking (15:00 hours) on each of the first 3 days postpartum, and on days 10 and 17 after calving; samples were collected daily thereafter until day 60 postpartum in most cases. In a few cases, milk sampling was terminated prior to 60 days when the milk progesterone profile indicated that postpartum ovulation had occurred on two occasions. The milk sampling procedure involved drawing a few strippings from each teat at mid-milking into a labelled plastic "whirl-pak" bag containing potassium dichromate preservative tablets. Samples were immediately cooled to 4°C and stored in the barn and transfered once a week to the laboratory where they were again stored at 4°C until assayed for progesterone. Samples were normally assayed within 2 weeks of collection.

In a preliminary experiment to confirm the parallelism between serum and milk progesterone levels, blood and milk samples were taken concurrently during afternoon milking, from three cows over a complete estrous cycle. Milk collection was by the same procedure described above, while blood collection was by vein-puncture of the coccigeal vein with a sterile hypodermic needle attached to a 7 ml vacutainer tube. Blood samples were stored overnight at 4°C and then centrifuged to obtain serum which was stored at -20°C until assayed for progesterone.

(ii) Progesterome Assay Procedures

Milk

The concentration of milk progesterone was determined by a RIA procedure essentially similar to that described by Heap et.al. (1973) in which the assay was conducted on non-extracted milk. Well mixed samples were first diluted 10-fold with phosphate buffer, pH 7.0 (1.0 g gelatine; 1.0 g NaN_3; 9.0 g NaCl; 5.38 g $NaH_2PO_4.H_2O$; 16.35 g $Na_2HPO_4.7H_2O$ in 1L distilled water). Portions of the diluted milk (0.1 ml) were then pipetted into a series of duplicate tubes and to each tube was added 0.5 ml phosphate buffer. At the same time, a series of duplicate standard tubes were prepared containing 0.5 ml solutions of known amounts of progesterone (Standard concentrations of 0, 25, 50, 100, 200, 400, 800 pg) in buffer and 0.1 ml milk with little or no detectable progesterone. Milk used for this purpose was taken from a non-cycling, non-pregnant lactating cow and diluted 10 fold with phosphate buffer. Portions (.1 ml) of a highly specific progesterone antiserum (Pool #37, generously supplied by Dr. G.D. Niswender, Colorado State University) that had been diluted with phosphate buffer solution was added to all standard and sample tubes. The antiserum was prepared by immunizing rabbits with progesterone-6-hemisuccinate-bovine serum albumin, and was used at an initial dilution of 1:3,000. Following the addition of the antiserum, 0.1 ml [1, 2-^{3}H] progesterone with a total activity of about 10,000 cpm was added to all tubes. All samples were then mixed on a vortex and incubated at 4°C for a minimum of 3 hours, or overnight. After incubation, 0.5 ml charcoal suspension, containing 250 mg washed charcoal (Norit A) and 25 mg dextran T70 per 100 ml phosphate buffer, was added at 4°C. The samples were then thoroughly mixed, incubated at 4°C for 20 mins., and centrifuged at 800 g for 10 mins. The supernatant containing the antibody-bound progesterone was decanted into disposable plastic vials and counted in 4 ml of "Aquasol" scintillation fluid, after a minimum of 3 hours equilibration at 4°C. The concentration of progesterone in the unknown milk samples were then extrapolated from the standard curve readings.

To determine intra- and inter-assay coefficient of variations, a pooled milk sample derived from cows at various times postpartum was included in the routine progesterone assays performed weekly. The mean progesterone concentration in the pooled sample from 11 independent assays was 23.9 $\pm$ 0.7 ng/ml with intra- and inter-assay coefficients of variation of 9.2% and 14.1%, respectively. The intra- and inter-assay coefficients of variation were calculated as described by Rodbard (1971).

Serum

Progesterone concentration in serum was determined by the RIA procedure modified by Yuthasastrakosol (1975), which was based on a method described earlier by Abraham et.al. (1971). The same highly specific antiserum (Pool #37), [1, 2-^{3}H] progesterone and the progesterone standard solutions used in the milk assays were employed in the serum assays. All serum samples were analysed in a single assay. The average percentage recovery of ^{3}H-progesterone after the extraction process was 76.8 $\pm$ .4 (n = 4) and was used to correct the results for procedural losses.

Interpretation of Milk Progesterone Data

After the completion of all assays, milk progesterone concentrations on days 1, 2, 3, 10, 17 and daily thereafter up to a maximum of 60 days postpartum were obtained for each animal. These postpartum progesterone profiles were used to determine the time of first postpartum ovulation, other subsequent ovulations, and the nature of postpartum ovarian activity based on the recognition that the pattern of progesterone changes in milk and blood reflect ovarian activity. Postpartum ovulation was presumed to have occurred when milk progesterone concentration increased progressively from levels lower than 2 ng/ml (usually < 1 ng/ml) to levels greater than 5 ng/ml within a period of 10 consecutive days.

Parameters Studied and Treatment Allocation

Part of the study involved investigating the effect of postpartum ovarian activity on subsequent fertility. The postpartum breeding record of each animal was therefore used to evaluate the interval from calving to conception, and the number of inseminations required per conception.

The other part of the study involved investigating the effect of season of calving, age at calving and level of milk production on the interval to first postpartum ovulation. Based on the milk progesterone profiles established earlier for each animal, the interval from calving to first postpartum ovulation was determined. The age and season of calving, and the average milk production for the first 4 weeks postpartum were also recorded for each animal. Treatment groups of spring plus summer (March 21 - August 21) and fall plus winter (August 22 - March 20) for season of calving; 2, 3 to 4, and 5 or more years for age at calving; low ($<$ 22.7 Kg/day), medium (22.7 - 31.8 Kg/day), and high ($>$ 31.8 Kg/day) for level of milk production were then defined. The postpartum interval to first ovulation data were subsequently allocated to these treatment groups to produce two randomized block design (RBD) tables comprising treatment groups of age at calving on one hand, milk production on the other and season of calving common to both.

Statistical Procedures

The statistical analyses performed were based on procedures described by Snedecor and Cochran (1967). Analysis of variance (Anova) for a one-way classification of means with unequal sample sizes was used to test the effect of postpartum ovarian activity on interval to conception. To test the effect of age, season and level of milk production on the interval to first postpartum ovulation, the Anova procedure for a two-way classification of means with unequal sample sizes was employed. The t-test was used to detect significant differences between seasonal means, while differences in age and level of milk production treatment means were tested by the Student - Newman-Keul's (SNK) test.

RESULTS

Relationship Between Serum and Milk Progesterone Concentrations During the Normal Estrous Cycle

The changes in mean serum and milk concentration of progesterone of three cows during the estrous cycle are shown in Figure 1. The general pattern of change was similar in both biological fluids, but the milk progesterone concentrations were 3 to 4 times higher than their corresponding serum levels between days 5 and 17 of the cycle. As might be expected, milk progesterone concentration was low (< 1 ng/ml) on the day of first estrus (day 0); levels then began to increase steadily by day 2 of the cycle reaching, on day 5, a mean level of 6.7 ng/ml compared to a mean serum level of 2.2 ng/ml at this time. No blood samples were taken prior to day 5. Both serum and milk progesterone levels continued to rise after day 5 with the milk levels increasing more dramatically than serum levels. The mid-cycle concentrations obtained between days 10 and 15 showed small fluctuations in serum progesterone but a trend of increasing concentration in milk with mean peak values of 5.5 ng/ml and 21.5 ng/ml being seen on day 15 in serum and milk, respectively. The progesterone level in both serum and milk dropped precipitously after day 15 reaching levels lower than 1 ng/ml two days prior to the next estrus.

The correlation between serum and milk concentrations

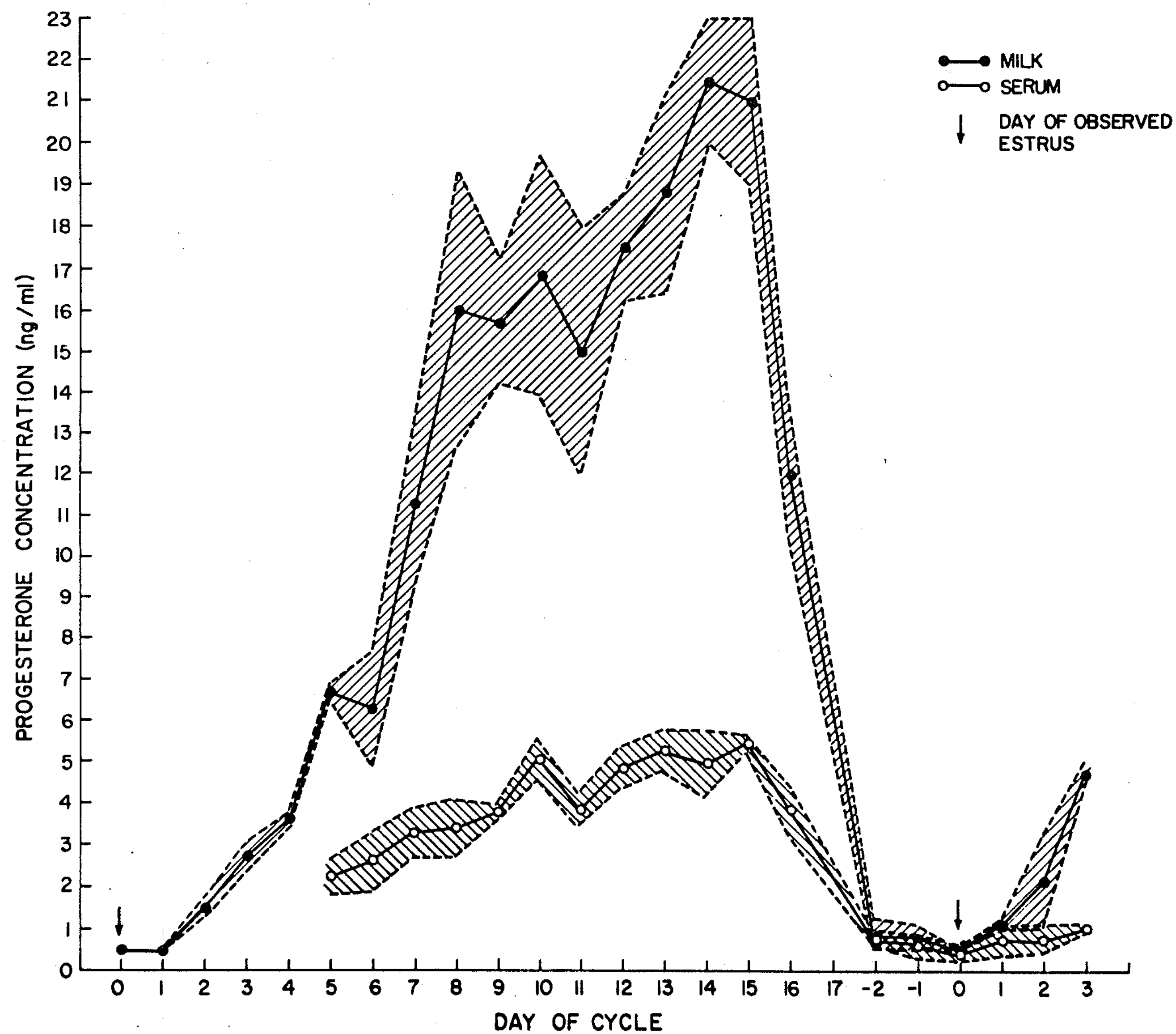

Figure 1. Composite milk and serum progesterone (mean $\pm$ S.E) profiles for 3 cows during the normal estrous cycle.

of progesterone was found to be highly significant ($P < .01$) in all three animals. The correlation coefficients obtained were 0.91, 0.93 and 0.88.

Postpartum Ovarian Activity Based on Milk Progesterone Profile

The activity of the ovary from calving to a maximum of 60 days postpartum was examined for each of the 52 animals using their milk progesterone profiles. The concentration of milk progesterone during the first 3 days postpartum generally ranged from less than 1 ng/ml to as high as 9.7 ng/ml with a mean of 2.7 $\pm$ .4 ng/ml. A large proportion (69.4%) of the animals had average concentrations of 2 ng/ml or less. The concentration of milk progesterone on day 10 postpartum was similarly variable with a mean of 3.1 $\pm$ .5 ng/ml and was either comparable to or higher than the mean concentration of the first three days in 60.9% of all animals.

A comparison of the levels of milk progesterone on days 10 and 17 indicated that some type of ovarian activity had resumed in all animals by day 17 postpartum. The progesterone profiles however revealed that some of these animals exhibited abnormal ovarian activity at one time or another during the period from calving to 60 days postpartum. Judging from the concentration of milk progesterone, 31 of the 52 animals did not appear to exhibit any abnormal ovarian activity prior to the first postpartum ovulation. The postpartum milk progesterone profiles for four of such animals are shown in Figures 2 and 3. In these animals, the concentration of milk progesterone generally remained low (3 ng/ml or less) after day 3 postpartum except for a transient rise that generally occurred about 4 to 7 days prior to the first ovulation. In 17 other animals, milk progesterone concentration was much higher than 3 ng/ml (higher than 40 ng/ml in some animals) prior to first ovulation for a prolonged and variable period of time which did not extend beyond 30 days postpartum. This may be an indication of either luteinization of developing follicles or the development of cystic ovaries which were resolved spontaneously. All but one of these animals calved either in the fall or the winter.

The concentration of milk progesterone at the presumed first ovulation generally ranged from less than 1 ng/ml to 3 ng/ml. The minimum detectable level using the assay was 1 ng/ml and the progesterone concentration at the first ovulation was equal to or lower than this value in 63.5% (33 of 52) of the animals. The levels of progesterone secreted during the entire first postpartum cycle, as indicated by mid-cycle peak levels, varied from animal to animal. As demonstrated in Figure 2, the mid-cycle peak level of progesterone was about 50 ng/ml for Leora compared to only about 10 ng/ml for Elsie. On the other hand, both Valerie and Lenora (Fig. 3a and b) had mid-cycle peak progesterone levels

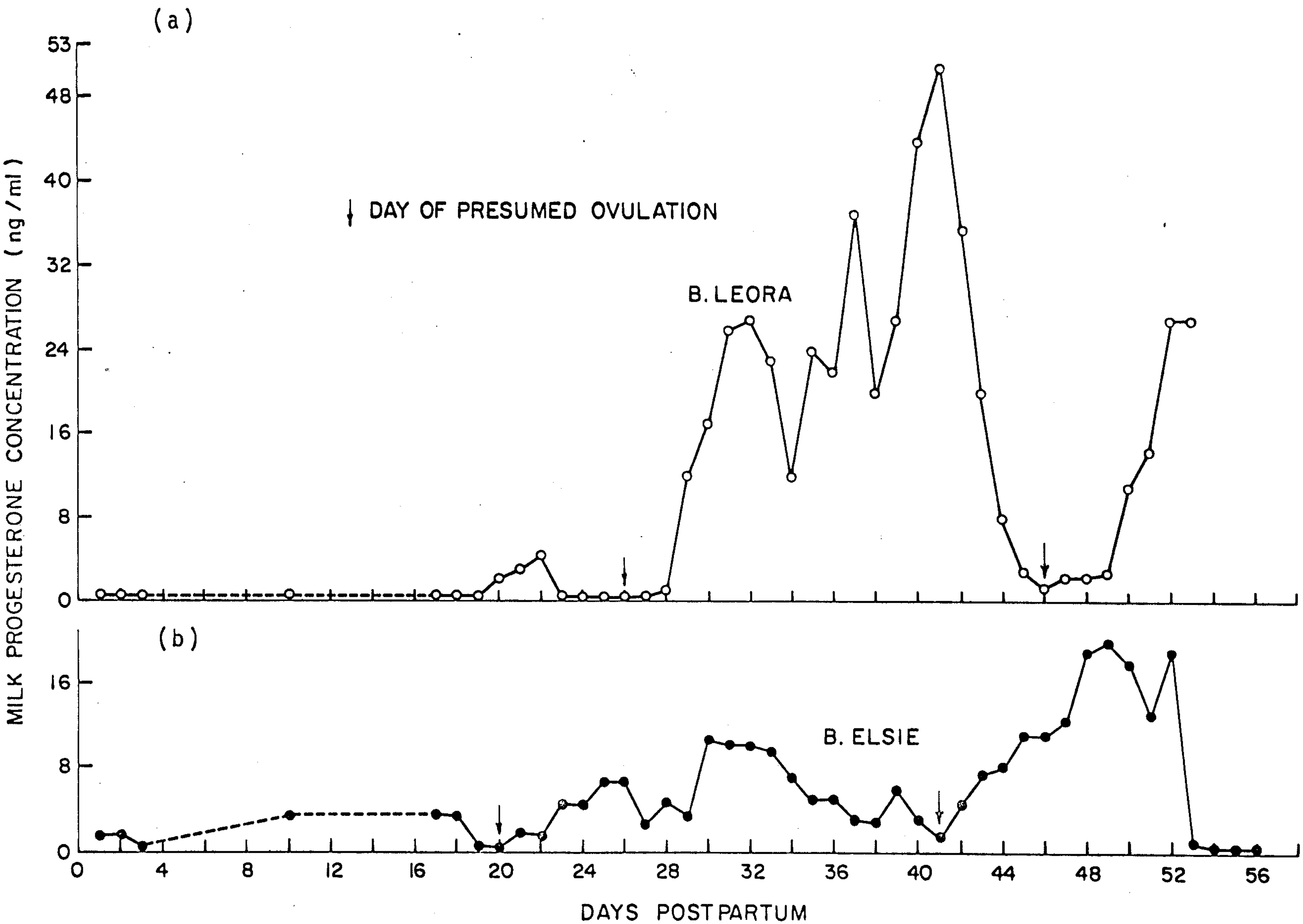

Figure 2. Postpartum milk progesterone profiles for two representative cows, B. Leora (a) and B. Elsie (b), exhibiting normal ovarian activity. No milk samples were taken between days 3 to 10 and 10 to 17 postpartum.

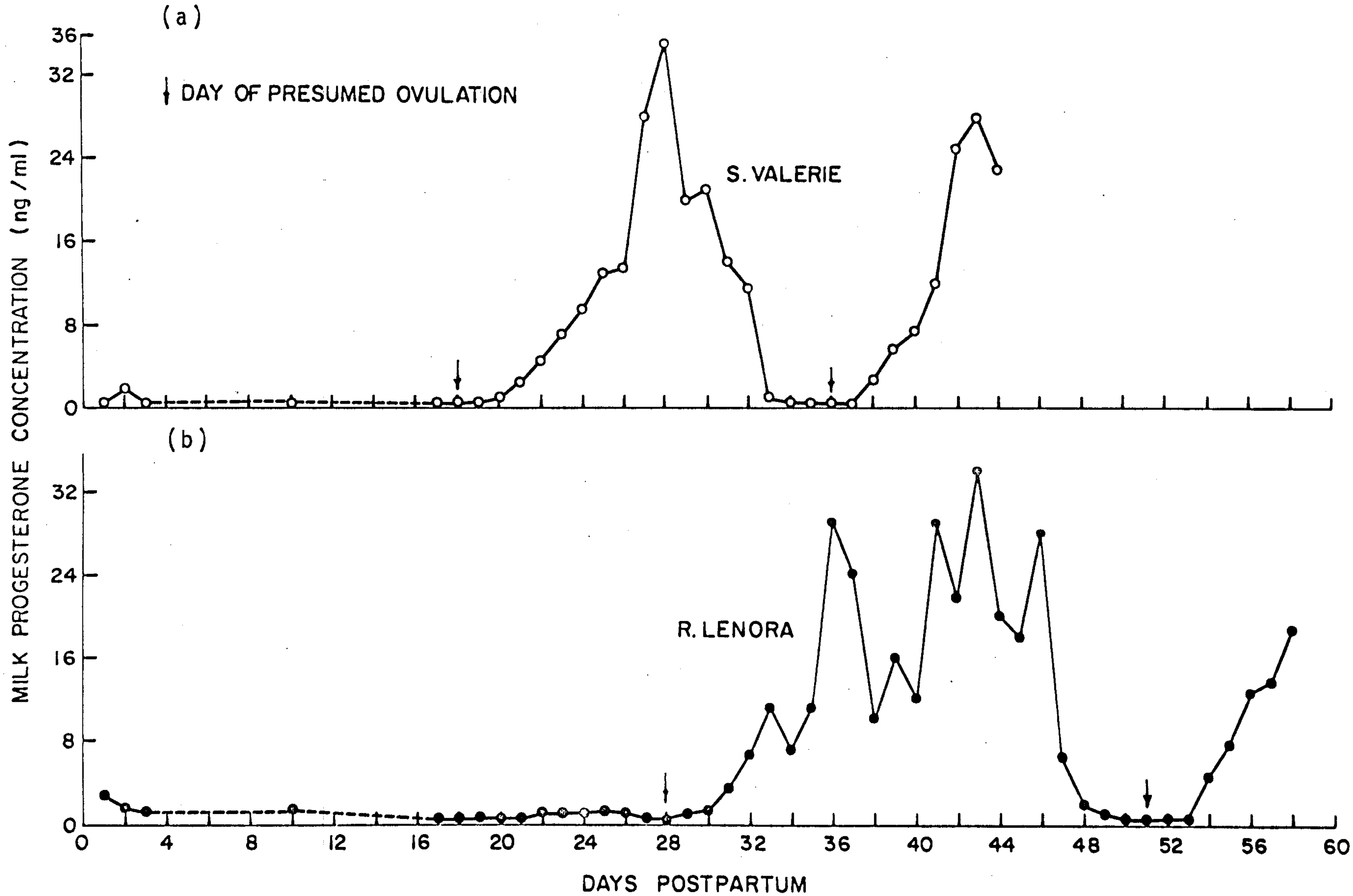

Figure 3. Postpartum milk progesterone profiles for two representative cows, S. Valerie (a) and R. Lenora (b), exhibiting normal ovarian activity. No milk samples were taken between days 3 to 10 and 10 to 17 postpartum.

of about 35 ng/ml. Levels as high as 60 ng/ml and above were seen in six animals. Although milk progesterone profiles covering the entire 60-day postpartum period were obtained for 77% of all the animals, only seven had two distinct ovarian cycles within this period. In these animals the levels of milk progesterone tended to be higher during the second cycle than during the first. This is demonstrated by the progesterone profile of Elsie (Fig. 2b) in which peak mid-cycle progesterone concentration of the second cycle was 20 ng/ml compared to only about 10 ng/ml in the first cycle.

Figure 4 depicts the composite milk progesterone profile during the first postpartum ovarian cycle of 22 animals that had no abnormal post-calving ovarian activity. A transient rise in milk progesterone which began 7 days prior to the first presumed ovulation and peaked at day -4 at $3.0 \pm .6$ ng/ml is clearly demonstrated. The concentration of milk progesterone then declined and at the time of presumed ovulation was less than 1 ng/ml. Milk progesterone concentration then rose sharply and steadily reaching a mean level of 25.5 ± 2.4 ng/ml by day 9 of the cycle. For the next 7 days, the mean mid-cycle levels fluctuated slightly between 24 and 26 ng/ml. A drop in milk progesterone concentration occurred after day 16, reaching mean levels of $1 \pm .2$ ng/ml 4 days later when the next ovulation was presumed to have occurred. The concentration of progesterone in the milk began to rise again after this ovulation.

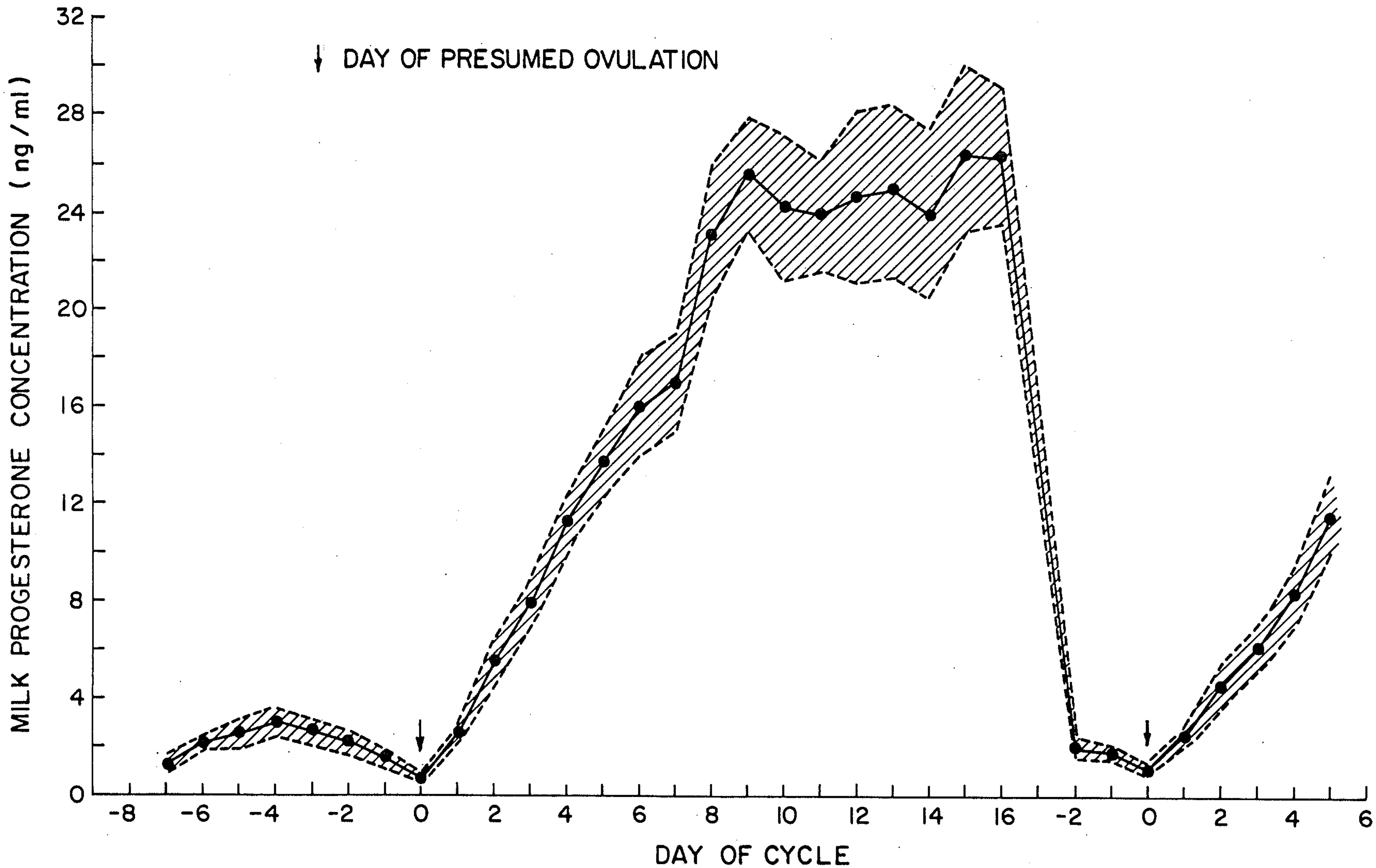

Figure 4. Composite milk progesterone (mean ± S.E) profile during the first postpartum ovarian cycle for 22 heifers and cows exhibiting normal ovarian activity.

The length of the first postpartum cycle was variable. Based on the milk progesterone profiles, the shortest and longest first postpartum cycles among animals with no apparent abnormal ovarian activity were 14 and 24 days, respectively. The average length of the first cycle for these animals was 20 days which is only a day shorter than the average of 21 days which is generally accepted for the normal cycle of the cow. Among the seven animals which had two distinct ovarian cycles by day 60 postpartum, the length of the second cycle in comparison to that of the first was longer by an average of 3.3 days in four; the same in one; and shorter by an average of 1.5 days in two animals. For this group of animals, the length of the second cycle ranged from 18 to 22 days.

Postpartum breeding data was available for a total of 41 animals which had gone through at least one complete ovarian cycle by 60 days postpartum and/or had a progesterone profile covering the entire 60-day postpartum period. Nineteen of these animals exhibited various degrees and types of abnormal ovarian activities which were reflected in their milk progesterone profiles. One animal in this group (A. Leo, Fig. 5b) had what is suggested to be hypo-ovarian activity, as evidenced by low milk progesterone concentrations which fluctuated from less than 1 ng/ml to a maximum of only 4 ng/ml throughout the 60-day period. There was failure of CL regression after the first presumed ovulation in six other animals. The progesterone profile of M. Queen (Fig. 5a)

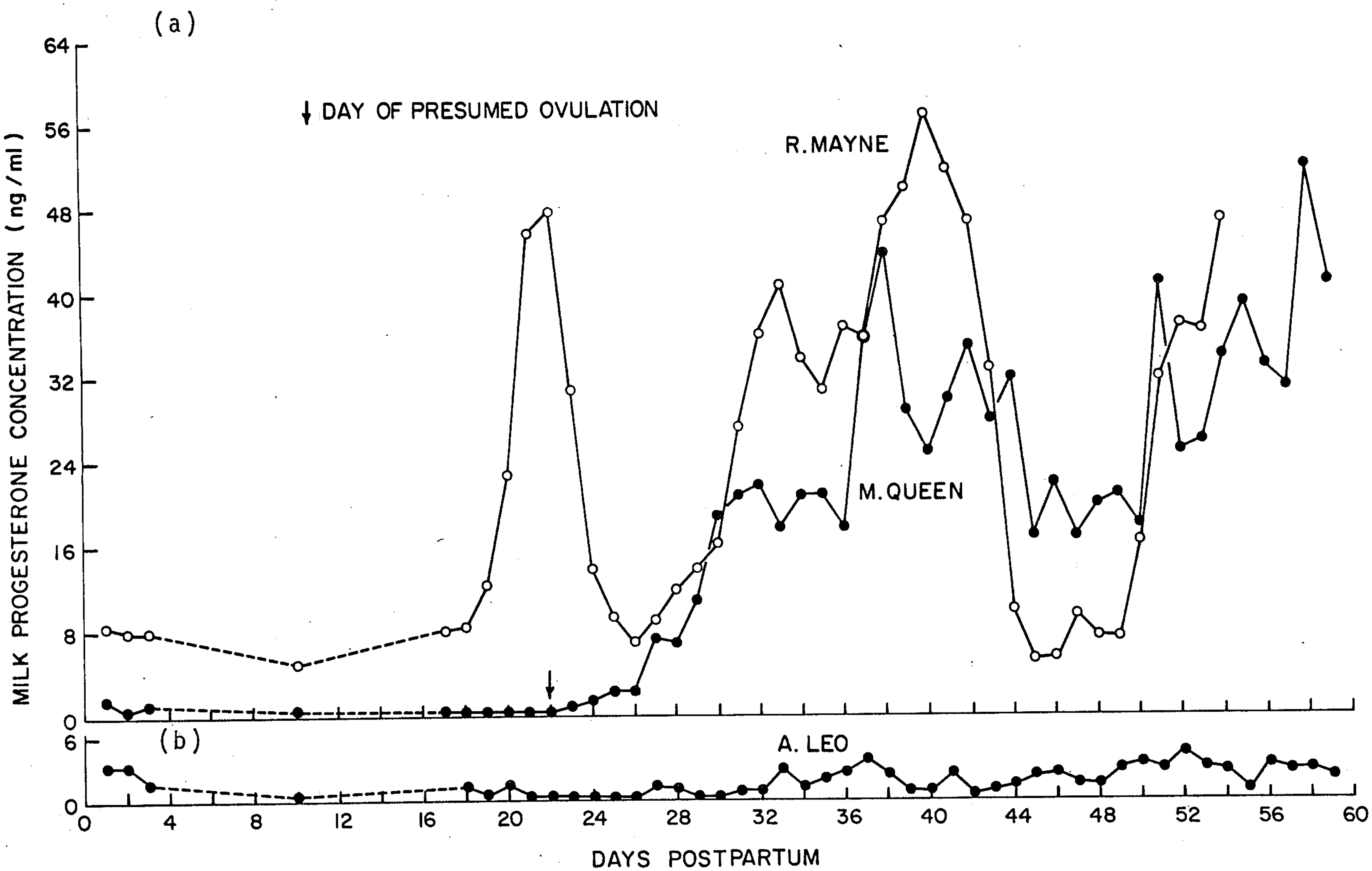

Figure 5. Postpartum milk progesterone profiles for three animals exhibiting abnormal ovarian activity: A Leo-hypo-ovarian activity; M. Queen - failure of CL regression after first ovulation; R. Mayne - high progesterone levels with no apparent ovulation prior to day 60.

demonstrates what might be considered to be the typical situation in these animals. After the first ovulation, the progesterone concentration in the milk rose sharply, as in a normal cycle, but the precipitous drop in progesterone that usually occurs on day 17 or 18 of a normal cycle, leading to the next ovulation, did not occur in these animals. Instead milk progesterone concentration fluctuated at levels higher than 10 ng/ml throughout the remainder of the postpartum period. In three animals, the concentration of milk progesterone was high (> 5 ng/ml) either right from day 1 postpartum or by day 17, and fluctuated at high levels thereafter with no apparent ovulation until after day 50 to 60. The progesterone profiles of two of these animals are depicted in Figures 5a (R. Mayne) and 6 (S. Lady). In R. Mayne, the concentration of milk progesterone dropped drastically on two occasions (days 26 and 45) but never reached the typical low ovulatory levels of less than 1 ng/ml usually observed in the other animals that were considered to be normal. The nature of the progesterone profiles in these animals suggested an incidence of either ovarian cysts or luteinization of growing follicles for a prolonged period of time. The type of ovarian abnormality exhibited by the largest number of animals (9) was an extended first postpartum cycle length (beyond 24 days) as demonstrated in the progesterone profile of S. Vision in figure 6. The length of the first cycle in these animals ranged from 26 to 39 days. This is an indication of a delay in CL regression

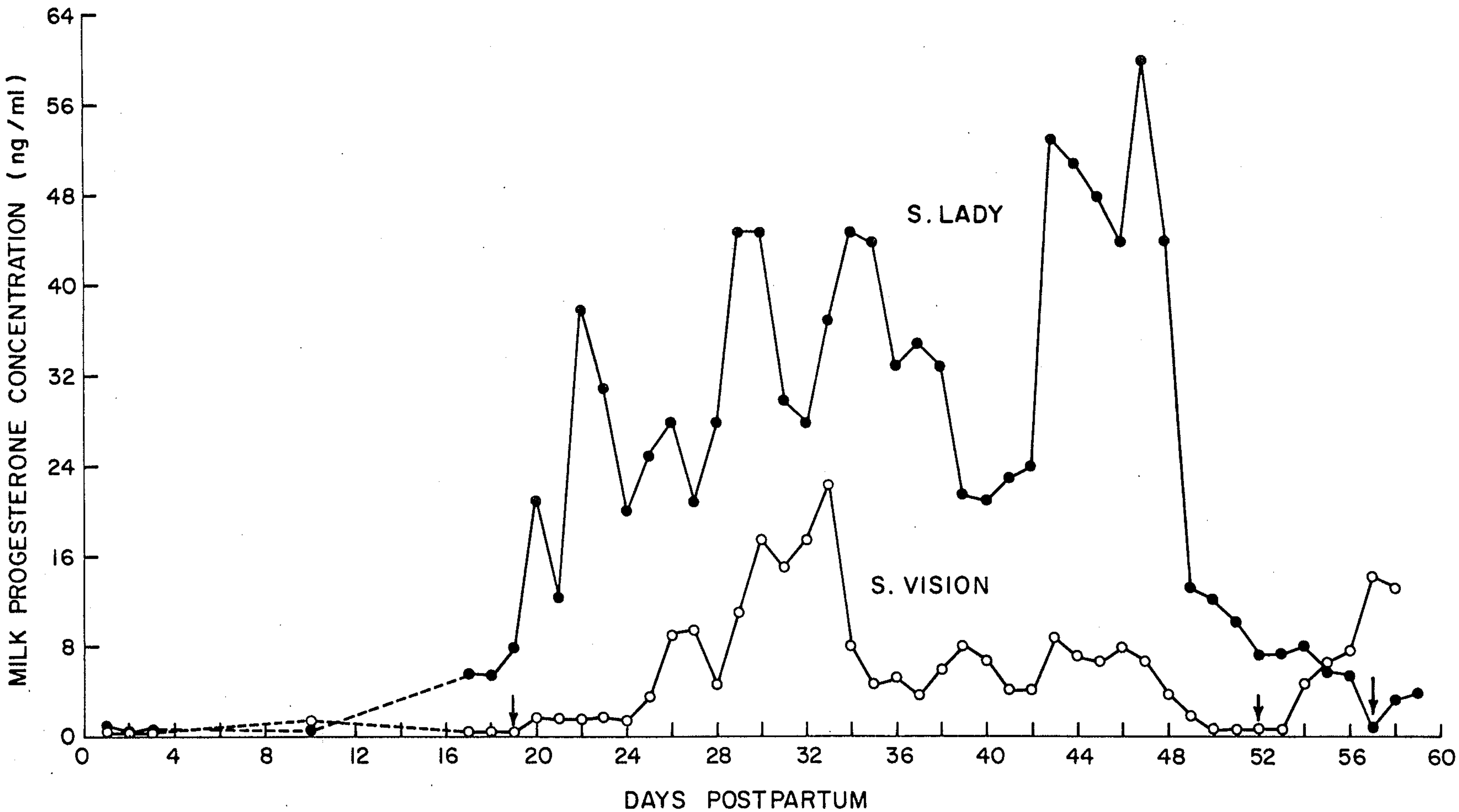

Figure 6. Postpartum milk progesterone profiles for two animals exhibiting abnormal ovarian activities: S. Lady - high progesterone levels with no apparent ovulation until day 57; S. Vision - Extended first postpartum cycle length (33 days).

which occurs normally on the 17th or 18th day of the cycle. The normal range of cycle length in cattle is generally accepted to be 17 to 24 days (Heap et.al. 1976).

The remaining 22 of the 41 animals which had postpartum breeding data, had at least one normal ovarian cycle between parturition and day 60 postpartum and none of the observed ovarian abnormalities. These animals were therefore classified as having normal postpartum ovarian activity. As shown in Table 1, the mean interval from calving to conception tended to be longer for animals exhibiting some degree of ovarian abnormality (141 $\pm$ 50 days) compared to those which had normal ovarian activity (114 $\pm$ 36 days), but this difference was not statistically significant ($P > .05$). In addition, the proportion of animals that became pregnant by 85 days postpartum was higher for the animals with normal ovarian activity (22.7%) than for those with ovarian abnormalities (15.8%). By 150 days postpartum, 77.3% of the "normal" animals had become pregnant compared to 57.9% of the "abnormals". The average number of inseminations required per conception was however comparable in the two groups.

In Table 2, the extent to which the various types of abnormalities affected the interval to postpartum conception are shown. Animals in which the first postpartum ovulation was delayed until after day 50, had the longest mean interval of 174.8 days to conception. This was followed by 147 days for animals with an extended first postpartum cycle length

TABLE 1: Effect of Ovarian Activity on the Interval From Calving to Conception

Ovarian Activity	No. of Animals	Interval from Calving to Conception (days) MEAN $\pm$ SE	RANGE	% Animals Pregnant by Various Days Postpartum 85	100	120	150	No. of Inseminations Per Conception
Normal	22	114 ± 36	60-200	22.7	31.8	63.6	77.3	1.6
Abnormal	19	141 ± 50	76-231	15.8	26.3	47.4	57.9	1.7

TABLE 2: Interval to Postpartum Conception as Affected by Type of Presumed Ovarian Abnormality

Type of Ovarian Abnormality	% of Total Abnormalities	Mean Interval to Conception (days)
1) High progesterone levels with no apparent ovulation until after day 50-60 postpartum	15.8	174.8
2) Extended first postpartum cycle length (26-39 days)	47.4	147.0
3) Failure of CL regression after first ovulation	31.6	128.0
4) Hypo-ovarian activity	5.2	97.0

and 128 days for those in which there was a failure of CL regression after the first ovulation. The animal with hypo-ovarian activity had a relatively short mean interval to conception of 97 days.

Interval From Calving to First Ovulation: Effect of Age, Season of Calving and Level of Milk Production During the Early Postpartum Period

Both the age of the animal and season of calving affected the interval from parturition to first postpartum ovulation based on the milk progesterone profile obtained for each animal. As shown in Figure 7a and b, the mean interval from calving to first ovulation was longer for animals calving in fall and winter compared to those calving in spring and summer in all three age groups. Mean interval to first ovulation was significantly ($P < .01$, t-test) longer in 2-year-old heifers which calved in fall and winter (30.5 ± 1.6 days) than in heifers which calved in spring and summer (24.2 ± 2.6 days). Similarly, cows aged 3 to 4 years, which calved in fall and winter, had a significantly ($P < .01$) longer interval to first ovulation (25.3 ± 1.7 days) than those which calved in spring and summer (22.8 ± 1.6 days). As well, among the cows aged 5 years or more, the interval to first ovulation was significantly ($P < .01$) longer by an average of 6.3 days for the ones that calved in fall and winter over those which calved in spring and summer. Although there was no significant interaction between age and season of calving, the differences between spring-summer and fall-winter calvings in terms of

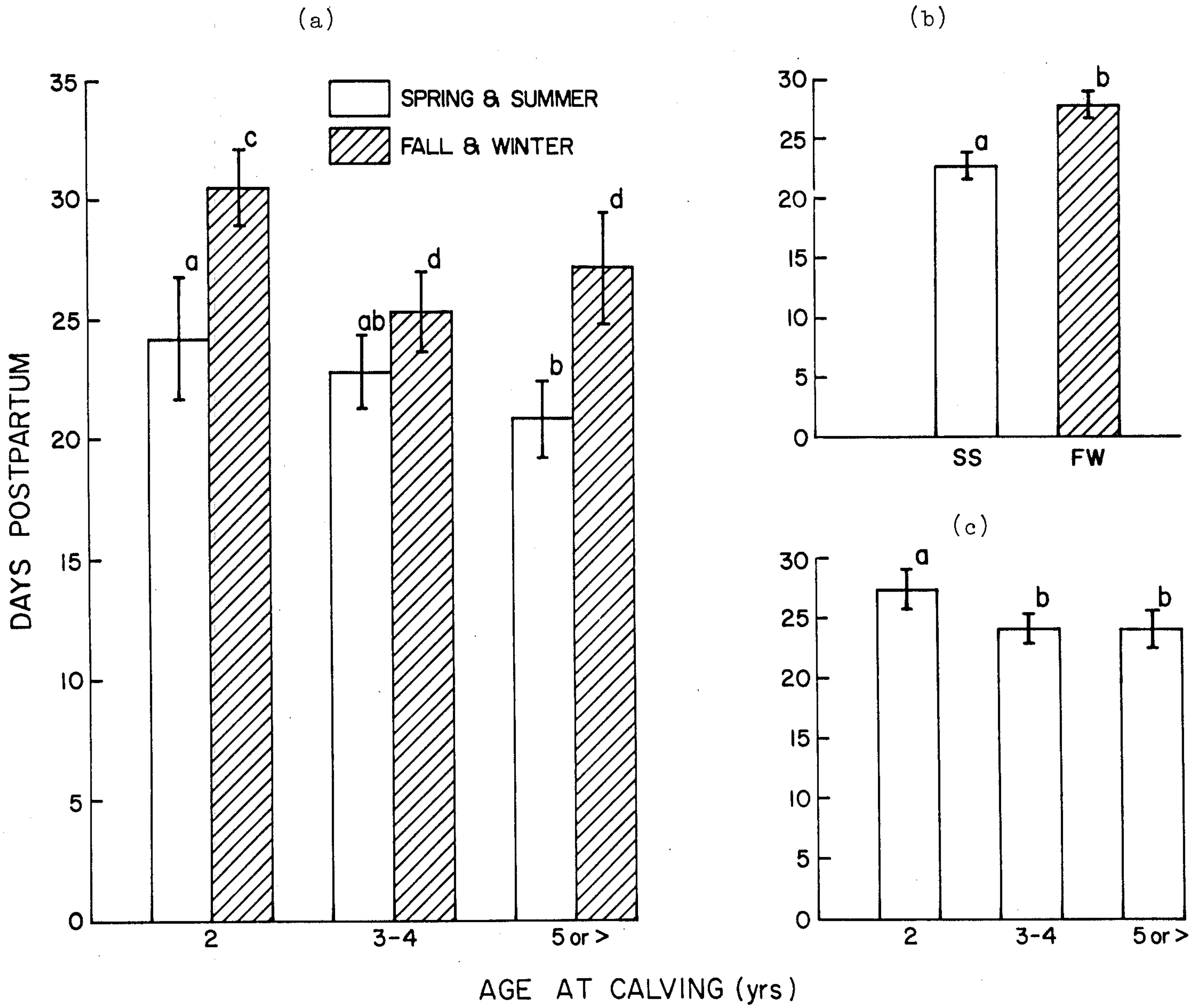

Figure 7. Effect of season and age (a), season (b) and age (c) at calving on the interval from parturition to first ovulation. Bars with different superscript letters are significantly different. ($P < .05$). In (a) the comparisons are between corresponding means within seasonal groups (SNK test) and age groups (t-test).

the interval to first ovulation was smallest in the cows aged 3 to 4 years. In both spring-summer and fall-winter however, the mean interval from calving to first ovulation was significantly ($P < .05$) longer in the 2-year-old heifers (Fig. 7a). There was no significant difference in the interval to first ovulation between the 3 to 4-year-olds and the cows aged 5 years or more. This is further demonstrated in Figure 7c which features a combination of the data from all four seasons.

The effect of the level of milk production during the first 4 weeks postpartum and season of calving are presented in Figure 8. Level of milk production in the early post-partum period did not affect the mean interval to first ovulation. For animals that calved in spring and summer, the mean interval from calving to first ovulation for the low, medium, and high milk producers was 24 ± 2.0, 23 ± 1.6 and 18.8 ± 1.1 days, respectively, but these differences were not significant ($P > .10$) (Figure 8a). Similarly, mean intervals to first ovulation were not significantly different between low, medium and high producers that calved in fall and winter. However, among the high producers, the mean interval to first ovulation was significantly ($P < .05$) longer for animals which calved in fall and winter (28.5 ± 1.5 days) compared to those which calved in spring and summer (18.8 ± 1.1 days). Differences in the mean interval to first ovulation between spring-summer and fall-winter calvings were not significant

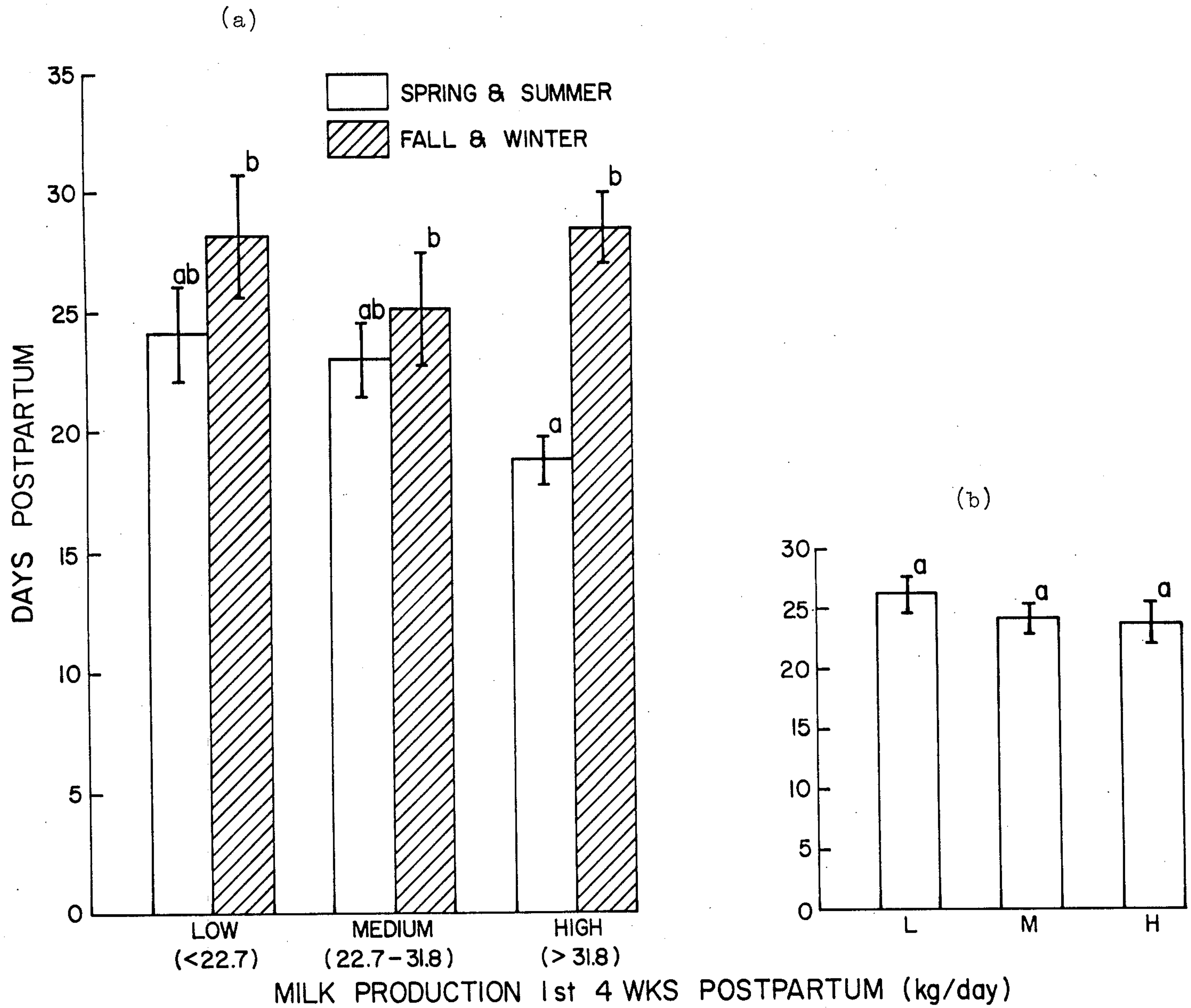

Figure 8. Effect of calving season and milk production (a) and milk production alone (b) (first 4 weeks postpartum) on the interval from parturition to first ovulation. Bars with different superscript letters are significantly different. ($P < .05$). In (a) the comparisons are between corresponding means within seasonal groups (SNK test) and milk production groups (t-test).

for the low and medium milk producers. When data from all the four seasons were combined the intervals to first ovulation, as shown in Figure 8b, were comparable for the low, medium and high producers.

DISCUSSION

The validity of using changes in milk progesterone concentration as an index of ovarian activity is based, in part, on the parallelism between the levels of progesterone in milk and blood which has been observed by earlier investigators (Heap et.al., 1973; Mauer et.al., 1974; Gartland et.al., 1975; Dobson et.al., 1975). In the present study, the similarity in the pattern of progesterone changes throughout the estrous cycle which resulted in the highly significant correlation between the concentrations of progesterone in milk and serum support these earlier findings. Furthermore, the levels of progesterone in milk were three to four fold higher than their corresponding levels in serum between days 5 and 17 of the estrous cycle which is in agreement with the earlier reports (Matuszezak et.al., 1974; Schiavo et.al., 1975). In addition, the milk progesterone concentration of less than 1 ng/ml observed at the time of estrus in this study is similar to the levels reported by Hoffman et.al., (1976) and Pennington et.al. (1976) and comparable to the frequently reported levels in serum at estrus. The peak mid-cycle progesterone level of 5.5 ng/ml for serum is similar to levels reported by most workers (Hendricks et.al., 1970; Snook et.al., 1971; Christensen et.al., 1974; Appleyard and Cook, 1976). However, the peak mid-cycle level of 21.5 ng/ml in milk is higher than the frequently reported values of around 10 ng/ml (Dobson et.al., 1975) but comparable to the levels reported by Pennington et.al. (1976a). Although levels of progesterone are relatively higher in milk than in serum, the bovine mammary glands apparently do not synthesize or metabolize progesterone (Heap et.al., 1975b). Furthermore, progesterone excreted via milk represents no more than 3% of the production rate in goats (Heap et.al., 1975a) and cows (Heap et.al., 1975b). Therefore, the high levels of progesterone in milk has been attributed to a high concentration of the steroid in the lipid fraction of milk owing to the relatively greater solubility of progesterone in lipid than in aqueous media. Heap et.al. (1975b) have shown that about 80% of administered ^{3}H-progesterone entering milk was found in fat, 19% in casein and 1% in aqueous fractions of milk. A similar distribution was observed when ^{3}H-progesterone was added to cow's milk in vitro.

The generally low levels of milk progesterone observed during the first 3 days postpartum is consistent with an earlier observation that the CL of pregnancy is present but regressing between 2 to 4 days after parturition (Oxenreider, 1968). The levels of milk progesterone indicated that all the animals on this study had resumed ovarian activity by day 17 postpartum. It was however impossible to determine exactly when this ovarian activity was initiated since no progesterone determinations were made between days 3 to 10 and 10 to 17. Nevertheless the fact that 60.9% of the animals had, on day 10 postpartum, progesterone concentrations which were comparable to or higher than the mean level during the first 3 days, may suggest that at least some of these animals had resumed ovarian activity by that time. Previous reports have indicated that the suppression of ovarian activity after parturition does not last beyond 7 to 10 days (Wagner and Hansel, 1969; Morrow et.al., 1969 a,b).

The transient rise in milk progesterone concentration prior to first ovulation observed in the animals exhibiting no ovarian abnormalities is consistent with the findings of Lamming and Bulman (1976). The first ovulation postpartum is generally known to occur without estrus and is said to be followed by a shortened luteal lifespan. Indeed, Marion and Gier (1968) and Morrow et.al. (1966) have estimated the average length of the first postpartum cycle to be 15 days. In the present study, animals were only casually observed for signs of estrus at the time of milking, and none was found in heat at the first ovulation. Only one animal had a typically short first postpartum cycle length but contrary to the earlier reports (Morrow et.al., 1966; Marion and Gier, 1968; Erb et.al., 1971), the average length of the first cycle was comparable to the length of a normal cycle. Furthermore, in five of the seven animals which had two cycles, the length of the first cycle was either comparable to or longer than the second. The tendency for higher levels of progesterone in the second cycle of these animals support the observations of Edgerton and Hafs (1973) and Fernandes _et.al._ (1978) that average progesterone concentrations increase throughout the postpartum period. Although it was impossible to detect any ovulations prior to day 17 in this study, some may indeed have occurred since postpartum ovulation in the dairy cow has been estimated to occur anytime between days 10 to 45 (Casida 1971, Bulman, 1978).

The majority of the ovarian abnormalities which seemed to indicate either luteinization of developing follicles or development of follicular cysts occurred in the very early postpartum period, but they degenerated spontaneously before day 30. However, three similar abnormalities persisted beyond day 50 postpartum and resulted in the longest mean interval to conception. These findings thus provide further evidence for the assertions of Morrow _et.al._ (1966) and Marion and Gier (1968) that although cystic follicles may be direct causes of delayed postpartum estrus and/or ovulation, most of them resolve themselves without treatment. Furthermore, this type of abnormality occurred almost exclusively in fall and winter as had been reported by earlier investigators (Roberts, 1955; Morrow _et.al._, 1966; Marion and Gier, 1968). The next major type of abnormality was the failure of CL regression after first ovulation. This was spontaneously resolved before day 40 producing what amounted to an extended cycle length in six animals. Two of the ovarian abnormalities identified in this study - hypo-ovarian activity and persistent CL - were also observed by Lamming and Bulman (1976) through the use of similar milk progesterone profiles. On the average, the interval from calving to conception was longer in the animals with postpartum abnormalities although the difference in comparison to the "normal" animals was not significant. In accordance with management practices which may be similar on most commercial dairy farms, the detection of estrus and first postpartum insemination of animals on this study were done between days 60 to 75. The results reported here however indicate that, on the average most animals had their first postpartum ovulation before day 35. The use of postpartum milk progesterone profiles, as clearly demonstrated in this study, could, therefore help in the identification and possible treatment of ovarian abnormalities before day 60, thus shortening the interval to conception.

The interval from calving to first ovulation was affected by both the season of calving and the age of the animal. Calving in fall and winter in comparison to spring and summer produced longer intervals in all the age groups. This is similar to the observations of other workers including Buch et.al. (1955), Wiltbank and Cook (1958) and Moskalenko (1970). In agreement with the findings of Wiltbank and Cook (1958), the interval was significantly longer in 2-year-old heifers than in all other cows aged 3 years or more. This is, however, contrary to the findings of Kohli and Suri (1957) that the interval to first estrus was longest for cows aged 5 to 6 years and over 12 years. The insignificant differences in the interval to first ovulation due to level of milk production supports the suggestion of Oxenreider and Wagner (1971) that the level of milk production has only a limited effect on postpartum anestrus. However, the level of milk production appears to be compounded with the seasonal stress since the interval to first ovulation among the high milk producers was significantly longer for those that calved in fall and winter.

The results indicate that milk progesterone could be a useful monitor of ovarian activity and could be a useful adjunct in diagnosis of infertility problems. Further research is required to establish, in the case of postpartum infertility, the optimum period to obtain milk progesterone profiles. A 21-day profile, obtained possibly between day 30 to 55 postpartum, may be useful in providing information regarding the reproductive status of the animal.

SUMMARY OF RESULTS AND CONCLUSION

Below is a summary of the results obtained from this study and the conclusion that can be drawn based on these results:

1) There was a highly significant ($P < .01$ correlation between the levels of progesterone in blood and milk samples collected from three cows over an entire estrous cycle. The general pattern of progesterone change was similar in both fluids.

2) The concentration of progesterone at the time of ovulation was generally comparable in both serum and milk.

3) The determination of progesterone concentration in milk samples collected on days 1, 2, 3, 10, 17 and daily thereafter until day 60 postpartum was effective in monitoring ovarian activity. Four different types of ovarian abnormalities were identified using this technique: (1) high progesterone levels with no apparent ovulation until after day 50-60 postpartum, (2) extended first postpartum cycle length, (3) failure of CL regression after first ovulation, and (4) hypo-ovarian activity. These abnormalities resulted in a longer interval to conception.

4) Ovarian activity resumed by day 17 postpartum in all animals and on the average first ovulation occurred by

day 35 in the majority of the animals. A transient rise in milk progesterone occurred 4 to 7 days prior to this ovulation.

5) The length of the first postpartum cycle was comparable to that of a normal cycle. The level of progesterone secreted during this cycle was, however, lower than normal.

6) The interval to first ovulation was signifcantly longer when animals calved in fall and winter and in 2-year-old heifers. The level of milk production during the early postpartum period, however, did not affect this interval although it appears to be compounded with the effects of the fall and winter calving.

Based on the results obtained in this study, it can be concluded that the development and careful interpretation of a milk progesterone profile for the postpartum dairy cow or heifer is effective in monitoring ovarian activity during the early postpartum period.

APPENDIX I

TABLE 1: Concentration of Progesterone (ng/ml) in The Milk of Three Cows During the Estrous Cycle

Day of Cycle	COWS			Mean Prog. Concn.	S.E.M. (±)
	M. Queen	Leona	Lenora		
0	0.5	0.5	0.5	0.5	0
1	0.5	0.5	0.5	0.5	0
2	1.0	1.6	1.85	1.5	0.31
3	2.8	3.3	2.0	2.7	0.41
4	3.5	4.0	3.4	3.6	0.2
5	6.6	7.1	6.4	6.7	0.2
6	3.8	8.8	6.4	6.3	1.4
7	9.6	15.5	8.9	11.3	2.1
8	9.6	17.5	21.0	16.0	3.4
9	13.5	18.5	15.0	15.7	1.5
10	11.5	17.5	21.5	16.8	2.9
11	8.9	18.5	17.5	15.0	3.0
12	18.5	19.0	15.0	17.5	1.3
13	17.0	16.0	23.5	18.8	2.4
14	20.0	24.5	20.0	21.5	1.5
15	19.0	25.0	19.0	21.0	2.0
16	11.0	-	31.0	12.0	1.4
-3	5.0	-	15.2	-	-
-2	0.5	1.2	-	0.85	0.4
-1	0.5	1.0	-	0.75	0.3
0	0.5	0.5	-	0.5	0
1	1.2	1.1	-	1.1	0.1
2	3.2	1.0	-	2.1	1.1
3	4.8	4.5	-	4.7	0.2
Cycle Length (days)	21	19	-	21	

TABLE 2: Concentration of Progesterone (ng/ml) in The Serum of Three Cows During the Estrous Cycle

Day of Cycle	COWS			Mean Prog.	
	M. Queen	Leona	Lenora	Conc.	S.E.M. (±)
0	-	-	0.5	-	-
1	-	-	0.6	-	-
2	-	-	1.6	-	-
3	-	-	1.5	-	-
4	-	-	2.4	-	-
5	1.6	2.2	2.9	2.2	0.4
6	1.4	2.6	3.8	2.6	0.7
7	2.1	3.5	4.3	3.3	0.6
8	2.3	3.3	4.6	3.4	0.7
9	4.2	3.6	3.6	3.8	0.2
10	4.4	4.8	6.2	5.1	0.5
11	4.2	3.1	4.5	3.9	0.4
12	4.1	4.7	5.8	4.9	0.5
13	6.1	4.6	5.2	5.3	0.5
14	4.5	6.5	4.0	5.0	0.8
15	5.1	5.8	5.5	5.5	0.2
16	3.3	-	4.5	3.9	0.6
-3	1.7	-	6.1	-	-
-2	0.6	1.0	-	0.8	0.2
-1	0.3	0.9	-	0.6	0.3
0	0.2	0.5	-	0.4	0.2
1	0.4	1.1	-	0.7	0.4
2	0.5	1.0	-	0.7	0.3
3	0.9	1.0	-	1.0	0.1
Cycle Length (days)	21	19	-	21	

TABLE 3: Cows With Normal Postpartum Ovarian Activity As Monitored by Milk Progesterone Profile

Cows	Length of First Postpartum Cycle (days)	Interval to Conception (days)	# Inseminations Per Conception
B. Visigoth	22	108	1
B. Elsie	21	158	3
R. Lenora	22	116	2
S. Valerie	18	102	2
B. Leona	19	110	1
S. Gem	21	90	1
A. Joan	17	134	2
S. Lola	22	133	2
B. Vendette	21	112	2
M.K. Queen	20	71	1
R. Nora	21	80	1
R. Queen	22	60	1
K. Nora	19	161	1
M.W. Queen	19	73	1
M. Juanna	21	98	1
C. Ann	20	105	3
C. Rosie	21	61	1
B. Vendome	23	154	3
F. Lois	17	110	1
S. Kathy	22	121	1
Y. Primrose	24	158	1
R. Fusion	14	200	3
Totals	446	2515	36
n = 22			
Mean	20	114 $\pm$ 36	1.6 $\pm$ .8

TABLE 4: Cows With Abnormal Postpartum Ovarian Activity As Monitored by Milk Progesterone Profile

Cow	Type of Abnormality Extended first postpartum cycle length	Interval to Conception (days)	# Inseminations Per Conception
Queen R.	26 days	116	2
P. Princess	27	217	3
B. Leona	28	170	3
F. Elsa	39	231	3
S. Vision	32	117	1
W. Queen	39	140	3
R. Melanie	30	79	1
B. Royal	39	76	1
J. Lady	34	177	1
	Failure of CL Regression After first ovulation		
M.M. Queen	"	106	1
L. Mark	"	87	1
A. Patricia	"	83	1
R. Joanne	"	114	3
M.C. Nora	"	180	1
S. Primette	"	198	3
	High Prog. Levels with no ovulation until after day 50		
S. Lady	"	153	2
C. Lois	"	140	1
R. Mayne	"	203	1
	Hypo-ovarian Activity		
A. Leo	"	97	1
Total (n = 19)		2684	33
Mean		141 $\pm$ 50	1.7 $\pm$ .9

TABLE 5: One-way Analysis of Variance with Unequal Sample Sizes: Effect of Postpartum Ovarian Activity on Interval to Conception

Source of Variation	df	SS	MS	f
Between Classes (type of Ovarian activity)	1	7401.4	7401.4	4.0 NS
Within Classes (error)	39	71641.0	1836.9	
TOTAL	40	79042.4		

TABLE 6: Interval From Calving to First Ovulation (Days): Effect of Age and Season of Calving

Season	Age at Calving (years) 2	3 - 4	5 or >	Mean
Spring & Summer	24.2 ± 2.6^{a}	22.8 ± 1.6^{ab}	20.8 ± 1.6^{b}	22.6 ± 1.1^{e}
n	6	10	6	22
Fall & Winter	30.5 ± 1.6^{c}	25.3 ± 1.7^{d}	27.1 ± 2.3^{d}	27.6 ± 1.2^{f}
n	6	9	9	24
Mean	27.4 ± 1.7^{g}	24.0 ± 1.2^{h}	24.0 ± 1.7^{h}	
n	12	19	15	

Horizontal and vertical means with different superscripts are statistically different (P < .05). The comparisons are between corresponding means within seasonal groups (SNK test) and age groups (t-test).

TABLE 7: Two-Way Analysis of Variance with Unequal Sample Sizes: Effect of Age and Season of Calving on Interval to First Ovulation

Source of Variation	df	SS	MS	f
Season of Calving	1	38.1	38.1	9.5*
Age at Calving	2	15.1	7.6	1.9+
Interaction	2	4.7	2.3	0.6
Within-cell Means	40		4.0	
Total	45	57.9		

*P < .05

+f was non-significant but SNK test showed significant differences

TABLE 8: Interval From Calving to First Ovulation (Days): Effect of Level of Milk Production and Season of Calving

	Level of Milk Production 1st 4 wks Postpartum			
	Low(<22.7 Kg/day)	Medium(22.7-31.8)	High(>31.8)	Mean
Spring & Summer	24.1 ± 2.0^{ab}	23.0 ± 1.6^{ab}	18.8 ± 1.1^{a}	22.0 ± 1.1
n	8	10	4	22
Fall & Winter	28.2 ± 2.6^{b}	25.1 ± 2.4^{b}	28.5 ± 1.5^{b}	27.3 ± 1.2
n	5	8	11	24
Mean	26.2 ± 1.6^{c}	24.1 ± 1.3^{c}	23.7 ± 1.7^{c}	
n	13	18	15	

Horizontal and vertical means with different superscripts are statistically different (P < .05). The comparisons are between corresponding means within seasonal groups (SNK test) and milk production groups (t-test).

TABLE 9: Two-Way Analysis of Variance With Unequal Sample Sizes (weighted Means): Effect of Level of Post-Partum Milk Production and Season of Calving on Interval to First Ovulation

Source of Variation	df	SS	MS	f
Level of Milk Production	2	37.4	18.7	0.6
Season of Calving	1	248.8	248.8	8.5*
Interaction	2	98.7	49.3	1.7
Within Sub-classes	40	1172.0	29.3	
Total	45	1556.8		

*P < .05

APPENDIX II

Levels of Milk Progesterone in 52 Post-parturient Heifers and Cows Which Calved in Four Different Seasons

SPRING (March 20th - June 20th, 1978)

B. VISIGOTH AGE: 2 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	2.0	42	1.5*
2	2.0	43	1.9
3	2.6	44	2.8
11	0.9	45	3.8
18	0.7	46	7.0
19	0.8*	47	11.0
20	1.5	48	14.5
21	7.5	49	17.5
22	9.5	50	24.0
23	7.0	51	27.5
24	10.0	52	31.5
25	10.0	53	38.5
26	9.0	54	31.0
27	5.5	55	30.0
28	5.0	56	-
29	5.1	57	19.5
30	5.2	58	21.5
31	10.0	59	35.0
32	5.0	60	14.5
33	4.0	61	18.5
34	4.0	62	1.5
35	4.5	63	1.5*
36	4.0		
37	3.9		
38	4.1		
39	5.0		
40	2.3		
41	3.9		

*Presumed ovulation

P. PRINCESS AGE: 3 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	0.5	42	16.5
2	1.3	43	15.0
3	1.4	44	19.0
11	0.5	45	9.5
18	0.7	46	1.0
19	0.7	47	1.0
20	-	48	1.0
21	3.6	49	0.5*
22	1.4*	50	1.0
23	3.8	51	1.0
24	10.0	52	8.0
25	7.5	53	9.5
26	7.5	54	12.5
27	10.0	55	18.0
28	12.5	56	20.5
29	15.0	57	21.0
30	16.5	58	27.0
31	13.5	59	35.0
32	29.0	60	45.0
33	26.0		
34	24.0		
35	26.0		
36	30.0		
37	26.0		
38	18.0		
39	23.0		
40	29.0		
41	17.0		

B. ELSIE AGE: 6 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Conc. (ng/ml)
1	1.9	39	5.9
2	1.8	40	3.0
3	0.5	41	1.5*
10	3.5	42	4.5
15	1.6	43	7.5
16	4.0	44	8.0
17	3.7	45	11.0
18	3.5	46	11.0
19	0.8	47	12.5
20	0.5*	48	19.0
21	1.9	49	20.0
22	1.6	50	18.0
23	4.7	51	13.0
24	4.5	52	19.0
25	6.8	53	1.0
26	6.8	54	<1.0
27	2.8	55	<1.0
28	4.8	56	<1.0
29	3.4	57	<1.0
30	10.5	58	<1.0
31	10.0	59	<1.0*
32	10.0	60	1.3
33	9.5		
34	7.0		
35	4.9		
36	5.0		
37	3.0		
38	2.9		

A.A. LEO AGE: 6 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Conc. (ng/ml)
1	3.4	42	1.0
2	3.3	43	1.0
3	2.0	44	1.4
10	<0.5	45	2.2
18	1.6	46	2.5
19	0.9	47	1.6
20	1.6	48	1.5
21	<0.5	49	3.0
22	<0.5	50	3.4
23	<0.5	51	3.0
24	<0.5	52	4.4
25	<0.5	53	3.2
26	<0.5	54	2.9
27	1.5	55	1.0
28	1.3	56	3.4
29	1.0	57	2.9
30	1.0	58	3.0
31	1.0	59	2.2
32	1.0	60	-
33	3.1		
34	1.4		
35	2.1		
36	2.9		
37	4.0		
38	2.6		
39	1.0		
40	1.0		
41	2.6		

B.T. QUEEN

AGE: 6 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	2.9	41	6.5
2	2.7	42	7.5
3	<0.5	43	9.5
10	2.0	44	11.5
17	14.8	45	35.0
18	16.0	46	27.5
19	23.0	47	45.0
20	24.0	48	32.5
21	33.5	49	12.0
22	35.0	50	12.0
23	37.0	51	16.0
24	50.0	52	21.0
25	50.0	53	14.5
26	50.0	54	11.0
27	32.5	55	14.5
28	15.0	56	20.5
29	17.0	57	24.5
30	14.5	58	16.5
31	12.5	59	25.0
32	4.5	60	21.0
33	3.6		
34	3.3		
35	2.9		
36	3.0		
37	5.0		
38	8.0		
39	4.1		
40	5.5		

Q. ROCKETTE

AGE: 11 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	2.0	41	13.5
2	<0.5	42	4.1
3	3.6	43	1.0
10	4.8	44	0.7
17	1.3	45	<0.5
18	0.9*	46	<0.5
19	1.1		
20	1.6		
21	2.3		
22	4.3		
23	8.3		
24	9.3		
25	9.5		
26	8.0		
27	12.0		
28	15.0		
29	16.0		
30	17.5		
31	17.0		
32	13.5		
33	11.5		
34	13.0		
35	9.8		
36	9.3		
37	11.0		
38	19.0		
39	18.5		
40	19.5		

SPRING 1979

M. ROSA AGE: 2 Yrs.

Day Postpartum	Milk Progesteron Conc. (ng/ml)
1	1.2
2	1.0
3	1.0
10	2.0
17	6.9
18	2.6
19	2.8
20	2.1
21	3.0
22	3.3
23	2.9
24	2.1
25	7.0
26	6.7
27	7.0
28	5.2
29	4.3
30	3.7
31	3.3
32	<1.0*
33	2.0
34	3.5
35	5.0

S. QUEEN AGE: 2 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	1.0
2	1.2
3	1.3
10	1.8
17	2.0
18	3.1
19	2.4
20	3.7
21	3.1
22	2.4
23	4.5
24	2.7
25	3.1
26	3.9
27	1.7*
28	4.1
29	7.2
30	7.2

SUMMER (June 21 - Sept. 22, 1978)

M. LADY

AGE: 2 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	1.0	41	23.0
2	0.5	42	24.0
3	0.8	43	53.0
10	0.5	44	51.0
17	5.8*	45	48.0
18	5.8	46	44.0
19	8.0	47	60.0
20	21.0	48	44.0
21	12.5	49	13.0
22	38.0	50	12.0
23	31.0	51	10.0
24	20.0	52	7.1
25	25.0	53	7.1
26	28.0	54	7.9
27	21.0	55	5.7
28	28.0	56	5.7
29	45.0	57	<1.0*
30	45.0	58	3.0
31	30.0	59	3.5
32	28.0	60	6.7
33	37.0		
34	45.0		
35	44.0		
36	33.0		
37	35.0		
38	33.0		
39	21.5		
40	21.0		

F. ELSA AGE: 2 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	2.2	41	9.5
2	1.9	42	5.7
3	1.8	43	7.5
10	1.0	44	11.0
17	4.4*	45	14.5
18	5.0	46	9.0
19	6.6	47	12.0
20	6.0	48	7.3
21	10.0	49	9.3
22	15.0	50	18.5
23	10.5	51	13.5
24	9.8	52	6.8
25	13.5	53	1.8
26	20.0	54	1.7
27	23.0	55	1.0
28	24.0	56	1.0
29	20.0	57	1.0*
30	22.0	58	1.0
31	21.0	59	7.5
32	10.0	60	7.0
33	12.0		
34	11.0		
35	11.5		
36	9.1		
37	10.5		
38	6.3		
39	4.3		
40	6.0		

S. VISION AGE: 2 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	1.0	41	4.0
2	1.0	42	4.6
3	1.0	43	8.5
10	1.5	44	6.9
17	1.0	45	6.3
18	1.0	46	7.8
19	1.0*	47	6.6
20	1.9	48	3.6
21	1.8	49	1.7
22	1.7	50	<1.0
23	1.8	51	<1.0**
24	1.6	52	<1.0
25	3.5	53	<1.0
26	9.1	54	4.7
27	9.5	55	6.4
28	4.7	56	7.3
29	11.0	57	14.0
30	17.5	58	13.0
31	15.0		
32	17.5		
33	22.5		
34	8.0		
35	4.7		
36	5.1		
37	3.7		
38	6.0		
39	8.0		
40	6.8		

**Observed estrus

M.K. QUEEN

AGE: 2 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Postpartum	Milk Progesterone Concn. (ng/ml)
1	1.0	42	31.0
2	1.0	43	45.0
3	1.0	44	34.0
11	1.0	45	30.0
18	4.7	46	18.5
19	8.5	47	30.0
20	12.0	48	4.5
21	14.0	49	1.0
22	16.5	50	1.1**
23	29.0	51	4.0
24	23.5	52	2.5
25	4.3	53	2.4
26	2.0	54	2.5
27	1.5	55	5.0
28	1.1	56	9.6
29	1.5	57	29.0
30	1.0*		
31	1.2		
32	3.5		
33	7.5		
34	16.0		
35	12.0		
36	23.0		
37	16.0		
38	39.0		
39	23.0		
40	20.5		
41	26.0		

M.B. LEORA AGE: 3 Yrs.

Day Postpartum	Milk Progesterone Conc. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	0.5	41	51.0
2	1.0	42	36.0
3	1.0	43	20.0
10	1.0	44	8.2
17	1.0	45	2.9
18	1.0	46	1.3*
19	1.0	47	2.2
20	2.1	48	2.1
21	3.0	49	2.7
22	4.2	50	11.0
23	<1.0	51	14.5
24	<1.0	52	27.0
25	<1.0	53	27.0
26	<1.0*	54	17.0
27	<1.0	55	23.0
28	1.0	56	27.0
29	12.0	57	20.0
30	17.0	58	37.0
31	26.0	59	28.0
32	27.0	60	26.0
33	23.0		
34	12.0		
35	24.0		
36	22.0		
37	37.0		
38	20.0		
39	27.0		
40	44.0		

S. GEM AGE: 3 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Postpartum	Milk Progesterone Concn. (ng/ml)
1	0.5	42	7.1
2	0.5	43	4.2
3	0.5	44	3.0
10	-	45	4.3
18	1.0	46	4.0
19	1.0	47	10.5
20	1.0*	48	15.0
21	6.2	49	14.0
22	8.7	50	19.0
23	14.0	51	18.0
24	11.0	52	22.0
25	20.0	53	30.0
26	25.0	54	23.0
27	22.0	55	40.0
28	26.0	56	26.0
29	21.0	57	31.0
30	-	58	22.0
31	23.0	59	22.0
32	24.0	60	15.0
33	20.0		
34	21.0		
35	14.0		
36	39.0		
37	26.0		
38	32.0		
39	35.0		
40	10.0		
41	3.9**		

S. VALERIE AGE: 4 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	0.5	41	12.0
2	2.0	42	25.0
3	1.0	43	28.0
10	1.0	44	23.0
11	1.0	45	27.0
18	1.0	46	33.0
19	1.0*	47	40.0
20	1.0	48	38.0
21	2.5	49	29.0
22	4.5	50	36.0
23	7.0	51	38.0
24	9.6	52	65.0
25	13.0	53	36.0
26	13.5	54	26.0
27	28.0	55	2.2
28	35.0	56	1.4*
29	20.0	57	5.1
30	21.0	58	2.8
31	14.0		
32	11.5		
33	1.0		
34	1.0		
35	<1.0		
36	<1.0		
37	<1.0*		
38	2.6		
39	5.7		
40	7.5		

B. LEONA AGE: 4 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	0.5	42	20.5
2	0.5	43	23.0
3	0.5	44	26.0
10	1.0	45	23.0
18	1.0	46	8.1
19	1.3	47	17.0
20	1.0	48	25.0
21	<1.0	49	16.0
22	<1.0	50	16.0
23	<1.0	51	25.0
24	<1.0	52	11.0
25	<1.0	53	14.0
26	<1.0	54	1.3
27	<1.0	55	1.0*
28	<1.0	56	1.0
29	<1.0*	57	4.3
30	1.4	58	4.3
31	2.1	59	4.0
32	12.0	60	-
33	12.0		
34	13.5		
35	10.0		
36	5.1		
37	11.0		
38	39.0		
39	18.0		
40	18.5		
41	22.0		

M.A. JOAN AGE: 4 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	1.0	41	24.0
2	1.0	42	25.0
3	-	43	33.0
10	1.0	44	20.0
17	2.3*	45	31.0
18	15.0	46	26.0
19	15.0	47	34.0
20	20.0	48	27.0
21	25.0	49	20.0
22	19.0	50	21.0
23	20.0	51	10.5
24	49.0	52	<1.0
25	39.0	53	<1.0
26	48.0	54	<1.0*
27	23.0	55	1.0
28	7.4	56	1.0
29	3.2	57	1.0
30	12.0	58	4.9
31	1.1	59	2.6
32	1.0	60	5.2
33	1.0		
34	1.0*		
35	2.5		
36	3.2		
37	5.7		
38	5.0		
39	10.5		
40	11.0		

S. LOLA AGE: 4 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	1.0	42	31.0
2	1.0	43	29.0
3	1.0	44	12.0
11	1.0	45	2.7
18	1.0	46	2.2
19	1.0	47	2.0
20	1.2	48	2.2
21	1.9	49	1.0*
22	6.0	50	3.3
23	5.7	51	7.7
24	4.8	52	10.0
25	1.9	53	17.0
26	2.5	54	24.0
27	1.7*	55	23.0
28	2.9	56	30.0
29	3.9	57	17.0
30	4.3	58	26.0
31	22.0	59	15.0
32	26.0	60	25.0
33	33.0		
34	37.0		
35	50.0		
36	44.0		
37	39.0		
38	24.0		
39	29.0		
40	20.0		
41	43.0		

L. MARK

AGE: 4 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Postpartum	Milk Progesterone Concn. (ng/ml)
1	1.0	42	16.0
2	1.0	43	10.5
3	1.0	44	26.0
11	1.0	45	27.0
18	1.0	46	25.0
19	1.0*	47	25.0
20	1.0	48	24.0
21	1.4	49	25.0
22	1.9	50	27.0
23	7.5	51	19.0
24	9.2	52	16.5
25	11.0	53	16.5
26	16.5	54	19.5
27	19.0	55	15.5
28	6.8	56	24.5
29	28.0	57	29.5
30	28.0	58	16.0
31	29.0	59	20.5
32	32.0	60	21.0
33	30.0		
34	23.0		
35	24.0		
36	42.0		
37	15.0		
38	8.5		
39	19.0		
40	12.5		
41	12.5		

A. LENETTE AGE: 4 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn.(ng/ml)
1	1.0	41	32.0
2	1.0	42	28.0
3	1.0	43	16.5
11	1.0	44	1.0
17	1.5	45	1.0
18	2.0	46	<1.0
19	1.2	47	<1.0
20	1.0	48	1.0
21	1.0	49	<1.0*
22	4.3	50	1.5
23	2.7	51	1.9
24	2.5	52	3.8
25	2.3	53	3.9
26	1.8	54	10.5
27	2.3	55	10.5
28	3.2	56	12.5
29	3.0	57	16.0
30	4.5	58	19.0
31	1.0*	59	13.5
32	2.3		
33	8.2		
34	15.5		
35	16.0		
36	20.0		
37	31.0		
38	31.0		
39	28.0		
40	36.0		

B. VENDETTE AGE: 4 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Postpartum	Milk Progesterone Concn. (ng/ml)
1	1.1	42	16.5
2	2.0	43	15.0
3	1.8		
11	1.0		
18	4.8*		
19	10.0		
20	15.0		
21	23.0		
22	25.0		
23	23.0		
24	25.0		
25	29.0		
26	33.0		
27	35.0		
28	39.0		
29	35.0		
30	39.0		
31	39.0		
32	44.0		
33	24.0		
34	8.0		
35	2.5		
36	3.1		
37	1.5		
38	2.5		
39	1.0*		
40	1.0		
41	2.1		

M.R. LENORA AGE: 5 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	3.0	41	29.0
2	1.6	42	22.0
3	1.2	43	34.0
10	1.4	44	20.0
17	1.0	45	18.0
18	1.0	46	28.0
19	1.0	47	6.3
20	1.0	48	1.9
21	1.0	49	1.0
22	1.0	50	<1.0
23	1.1	51	<1.0*
24	1.1	52	<1.0
25	1.2	53	<1.0
26	1.0	54	4.5
27	1.0	55	7.5
28	1.0*	56	12.5
29	1.0	57	13.5
30	1.3	58	18.5
31	3.4	59	36.0
32	6.5	60	20.0
33	11.0		
34	7.0		
35	11.0		
36	29.0		
37	24.0		
38	10.0		
39	16.0		
40	12.0		

M.M. QUEEN | AGE: 5 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	1.6	41	30.0
2	0.5	42	35.0
3	1.1	43	28.0
10	1.0	44	32.0
17	1.0	45	17.0
18	1.0	46	22.0
19	1.0	47	17.0
20	1.0	48	20.0
21	1.0	49	21.0
22	1.0	50	18.0
23	1.0*	51	41.0
24	1.6	52	25.0
25	2.6	53	26.0
26	2.5	54	34.0
27	7.5	55	39.0
28	7.0	56	33.0
29	11.0	57	31.0
30	19.0	58	52.0
31	21.0	59	41.0
32	22.0	60	51.0
33	18.0		
34	21.0		
35	21.0		
36	18.0		
37	36.0		
38	44.0		
39	29.0		
40	25.0		

R. NORA AGE: 6 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	1.0	42	5.5
2	1.0	43	9.0
3	1.0	44	16.0
12	1.0	45	13.5
18	2.1*	46	14.0
19	5.5	47	13.5
20	6.5	48	19.0
21	8.5	49	18.0
22	13.0	50	24.0
23	13.5	51	18.5
24	21.0	52	24.0
25	28.0	53	20.5
26	23.0	54	16.0
27	26.0	55	18.5
28	24.0	56	22.0
29	25.0	57	13.5
30	33.0	58	1.0
31	31.5	59	1.0
32	38.0	60	1.0*
33	34.0		
34	9.5		
35	4.2		
36	1.4		
37	2.7		
38	1.0		
39	1.1*		
40	3.3		
41	1.5		

W. QUEEN AGE: 7 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	1.0	41	19.0
2	1.0	42	28.0
3	1.0	43	22.0
10	1.0	44	22.0
17	3.7	45	21.0
18	3.7	46	35.0
19	2.5*	47	23.0
20	4.0	48	23.0
21	7.9	49	26.5
22	12.0	50	14.5
23	10.5	51	16.0
24	8.5	52	15.0
25	9.0	53	11.0
26	11.0	54	1.25
27	16.0	55	1.0
28	13.0	56	1.0
29	15.0	57	<1.0
30	20.0	58	<1.0
31	11.0	59	<1.0*
32	18.0	60	<1.0
33	28.0		
34	17.5		
35	21.0		
36	16.0		
37	18.5		
38	13.5		
39	14.0		
40	15.0		

FALL (Sept. 23 - Dec. 21, 1978)

K. NORA AGE: 2 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/nl)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	2.1	41	16.5
2	1.4	42	26.0
3	1.6	43	26.0
10	1.6	44	23.0
17	4.2	45	39.0
18	4.3	46	35.0
19	1.0	47	8.5
20	1.0	48	1.0
21	1.0	49	1.0
22	9.0	50	1.0**
23	9.2	51	11.0
24	7.2	52	10.5
25	1.0	53	12.0
26	5.1	54	14.5
27	3.0	55	12.0
28	3.0	56	18.0
29	1.0	57	19.0
30	<1.0	58	34.0
31	<1.0*	59	29.0
32	2.2	60	30.0
33	13.0		
34	15.0		
35	4.9		
36	9.8		
37	13.0		
38	16.0		
39	19.0		
40	17.0		

K. PATTY AGE: 2 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	1.0	41	10.5
2	1.0	42	9.6
3	-	43	9.0
10	1.9	44	20.0
17	1.0	45	19.5
18	1.0	46	17.5
19	5.1	47	18.0
20	5.9	48	14.0
21	2.0	49	15.0
22	1.9	50	16.5
23	1.0	51	17.0
24	2.1	52	11.0
25	1.1	53	14.0
26	13.5	54	1.0
27	7.2	55	<1.0
28	10.0	56	<1.0
29	13.5	57	<1.0*
30	11.5	58	1.0
31	11.0	59	1.0
32	13.5	60	6.4
33	1.2		
34	1.2		
35	1.0		
36	<1.0*		
37	4.5		
38	3.7		
39	-		
40	13.5		

M.C. NORA AGE: 2 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	-	42	4.5
2	-	43	5.5
3	-	44	6.4
10	6.0	45	3.5
18	5.4	46	2.8
19	6.9	47	2.4
20	6.6	48	5.9
21	5.4	49	6.4
22	3.5	50	2.8
23	5.1	51	3.6
24	4.1	52	4.0
25	1.9	53	8.1
26	2.2	54	12.0
27	1.0*	55	7.4
28	1.7	56	5.6
29	3.7	57	8.0
30	3.7	58	6.3
31	4.5	59	6.9
32	2.8	60	1.0
33	9.6		
34	7.0		
35	5.3		
36	6.5		
37	6.5		
38	6.3		
39	4.7		
40	2.6		
41	8.2		

J. LADY

AGE: 2 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	5.2	41	5.0
2	5.1	42	4.8
3	7.0	43	3.2
10	1.8	44	3.3
17	3.4	45	2.8
18	1.8	46	3.8
19	3.1	47	3.0
20	7.9	48	2.4
21	6.9	49	2.3
22	1.8	50	8.4
23	3.3	51	6.5
24	2.3	52	6.3
25	1.0*	53	8.8
26	1.1	54	6.3
27	1.7	55	6.5
28	1.6	56	6.3
29	7.0	57	1.0
30	2.8	58	<1.0
31	3.1	59	<1.0*
32	4.0	60	<1.0
33	4.5		
34	4.7		
35	12.0		
36	10.0		
37	6.1		
38	7.8		
39	8.5		
40	6.8		

C. LOIS AGE: 3 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	1.0	43	53.0
2	1.0	44	33.0
3	1.0	45	30.0
11	5.0	46	29.0
19	18.5	47	27.0
20	37.0	48	22.0
21	40.0	49	19.0
22	40.0	50	<1.0
23	31.0	51	<1.0*
24	36.0	52	1.0
25	23.0	53	1.0
26	11.5	54	1.2
27	11.5	55	2.7
28	10.5	56	6.3
29	11.0	57	4.5
30	9.3	58	8.3
31	7.7	59	12.0
32	7.0	60	40.0
33	10.5		
34	5.6		
35	12.5		
36	15.5		
37	15.6		
38	11.0		
39	8.9		
40	24.0		
41	27.0		
42	26.0		

P. BEATRICE AGE: 4 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	1.0	41	39.0
2	1.6	42	53.0
3	1.8	43	56.0
10	6.7	44	53.0
17	7.0	45	35.0
18	5.8	46	64.0
19	6.3	47	45.0
20	11.0	48	8.9
21	11.0	49	6.7
22	20.5	50	5.0
23	12.5	51	4.3
24	14.5	52	4.7
25	20.0	53	2.6*
26	16.5	54	5.9
27	9.8	55	7.6
28	11.5	56	9.6
29	3.0*	57	12.0
30	13.0	58	12.0
31	10.0	59	13.0
32	6.5	60	15.5
33	6.8		
34	8.8		
35	9.8		
36	14.5		
37	14.5		
38	15.0		
39	50.0		
40	53.0		

A. PATRICIA AGE: 5 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Porgesterone Concn. (ng/ml)
1	1.0	41	13.0
2	1.3	42	12.0
3	1.1	43	16.0
10	1.0	44	21.0
17	2.5	45	37.0
18	2.1	46	40.0
19	1.0	47	35.0
20	1.6	48	12.5
21	3.0	49	8.2
22	1.9	50	11.0
23	1.6	51	17.0
24	1.1	52	27.0
25	1.0	53	25.0
26	<1.0	54	31.5
27	<1.0	55	15.0
28	<1.0	56	13.0
29	<1.0	57	7.1
30	<1.0	58	11.5
31	<1.0*	59	10.5
32	4.3	60	3.7
33	3.6		
34	1.0		
35	1.0		
36	1.0		
37	2.5		
38	2.7		
39	6.2		
40	9.8		

M.W. QUEEN AGE: 5 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	-	41	21.5
2	-	42	17.5
3	-	43	25.0
10	7.1	44	25.5
17	<1.0*	45	26.0
18	<1.1	46	35.0
19	6.7	47	29.0
20	11.0	48	22.0
21	22.5	49	30.0
22	17.0	50	14.0
23	10.0	51	1.9
24	16.0	52	1.0
25	16.0	53	1.2
26	13.0	54	1.0*
27	12.5	55	3.8
28	21.0	56	5.3
29	31.0	57	6.5
30	15.5	58	2.2
31	12.0	59	7.6
32	3.6	60	6.9
33	5.0		
34	3.3		
35	2.6		
36	1.0**		
37	6.7		
38	8.6		
39	8.0		
40	17.5		

R. QUEEN   AGE: 6 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	1.0	41	6.7
2	1.0	42	14.0
3	1.0	43	11.5
10	1.0	44	21.0
17	11.5	45	28.0
18	14.0	46	32.0
19	14.5	47	27.5
20	13.0	48	39.0
21	31.0	49	36.0
22	9.6	50	25.0
23	24.0	51	18.5
24	29.0	52	34.0
25	35.0	53	31.5
26	21.0	54	31.0
27	25.0	55	11.5
28	27.0	56	16.0
29	12.0	57	16.0
30	4.9	58	2.5
31	3.0	59	1.4
32	1.4	60	1.0**
33	1.0		
34	<1.0		
35	<1.0		
36	<1.0		
37	<1.0*		
38	1.0		
39	1.1		
40	4.4		

R. JOANNE AGE: 6 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	10.5	41	5.8
2	8.0	42	8.9
3	7.0	43	12.0
10	6.6	44	10.5
17	5.1	45	9.6
18	5.1	46	7.0
19	4.6	47	10.5
20	2.5	48	12.0
21	3.9	49	12.5
22	3.2	50	9.0
23	1.5	51	9.8
24	1.6	52	6.4
25	1.6	53	6.0
26	12.5	54	8.5
27	-	55	10.5
28	10.0	56	8.3
29	1.0	57	6.5
30	<1.0*	58	22.5
31	3.5	59	17.0
32	2.3	60	13.5
33	2.4		
34	2.7		
35	4.7		
36	8.4		
37	10.0		
38	6.2		
39	8.4		
40	7.4		

M. FUSION AGE: 8 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	1.3	41	20.5
2	1.0	42	27.0
3	1.0	43	40.0
10	3.0	44	30.0
17	3.5	45	61.0
18	4.8	46	50.0
19	11.5	47	22.5
20	1.8*	48	13.0
21	5.6	49	29.0
22	9.6	50	45.0
23	16.0	51	9.6
24	15.0	52	7.3
25	13.0	53	8.5
26	8.2	54	2.9
27	13.0	55	2.2
28	4.8	56	1.5*
29	5.6	57	1.8
30	4.4	58	1.8
31	2.0	59	7.3
32	1.9	60	7.0
33	2.7		
34	1.7*		
35	2.2		
36	3.3		
37	4.4		
38	8.2		
39	17.0		
40	32.0		

WINTER (Dec. 22, 1978 - March 19, 1979)

M. JUANNA AGE: 2 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	1.5	42	3.8
2	2.8	43	2.5
3	3.7	44	4.0
11	-	45	3.9
18	4.5	46	3.9
19	8.0	47	14.0
20	3.8	48	1.0
21	3.3	49	1.0
22	3.5	50	1.0
23	3.6	51	<1.0
24	3.5	52	<1.0
25	1.3	53	<1.0*
26	1.7	54	<1.0
27	1.0	55	1.0
28	1.3	56	1.0
29	2.1	57	1.0
30	2.2	58	1.0
31	1.8	59	1.0
32	1.0*	60	3.9
33	1.1		
34	9.5		
35	8.4		
36	9.8		
37	9.5		
38	9.2		
39	7.4		
40	6.9		
41	2.4		

S. ROXANNE AGE: 2 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	5.4	41	33.5
2	8.1	42	33.5
3	13.0	43	39.0
10	3.4		
17	5.2		
18	8.7		
19	10.5		
20	5.1		
21	21.0		
22	33.0		
23	40.0		
24	55.0		
25	44.0		
26	58.0		
27	41.0		
28	57.0		
29	55.0		
30	12.5		
31	2.4		
32	2.1*		
33	5.7		
34	3.3		
35	3.0		
36	4.8		
37	8.9		
38	11.5		
39	26.5		
40	27.0		

S. PRIMETTE   AGE: 3 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	4.6	42	32.0
2	6.3	43	42.0
3	3.4	44	36.0
10	-	45	24.0
18	2.0*	46	22.0
19	2.2	47	8.0
20	8.7	48	8.4
21	10.0	49	10.5
22	10.0	50	5.1
23	8.0	51	8.6
24	5.8	52	9.5
25	9.7	53	11.0
26	10.0	54	15.5
27	5.0	55	11.5
28	6.5	56	15.5
29	8.5	57	18.0
30	12.0	58	21.0
31	12.0	59	19.5
32	12.5	60	17.5
33	15.0		
34	12.5		
35	14.5		
36	16.5		
37	22.5		
38	27.0		
39	25.0		
40	25.0		
41	25.0		

R. MELANIE AGE: 3 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	3.2	41	15.0
2	1.0	42	13.5
3	1.6	43	14.5
10	7.4	44	29.0
17	7.5	45	36.0
18	7.5	46	34.0
19	7.1	47	40.0
20	7.2	48	41.0
21	1.0	49	39.0
22	1.0	50	40.0
23	1.2	51	26.5
24	1.0	52	11.5
25	1.0	53	10.5
26	<1.0	54	6.0
27	<1.0*	55	4.8
28	5.3	56	2.1
29	3.9	57	1.6*
30	-	58	5.3
31	6.5	59	4.5
32	-	60	13.0
33	2.8		
34	11.5		
35	16.5		
36	16.5		
37	14.0		
38	13.0		
39	13.5		
40	15.0		

B. ROYAL

AGE: 3 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	10.0	41	29.0
2	9.5	42	29.0
3	9.5	43	32.0
10	6.5	44	35.0
17	1.0*	45	36.0
18	4.2	46	41.0
19	5.3	47	33.5
20	28.0	48	34.0
21	60.0	49	38.0
22	36.0	50	39.0
23	51.0	51	6.0
24	39.0	52	7.9
25	51.0	53	7.4
26	37.0	54	5.0
27	47.0	55	1.0
28	44.0	56	<1.0*
29	48.0	57	1.0
30	47.5	58	8.4
31	50.0	59	8.3
32	35.0	60	21.0
33	17.0		
34	14.0		
35.	11.0		
36	13.5		
37	14.0		
38	17.0		
39	16.5		
40	12.5		

R. MAYNE AGE: 3 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	8.7	41	52.0
2	8.0	42	47.0
3	8.0	43	33.0
10	5.0	44	10.0
17	8.2	45	5.2
18	8.7	46	5.7
19	12.5	47	9.5
20	23.0	48	7.5
21	46.0	49	7.6
22	48.0	50	16.5
23	31.0	51	32.0
24	14.0	52	37.0
25	9.5	53	36.5
26	7.2	54	47.0
27	9.3	55	19.5
28	12.0		
29	14.0		
30	16.5		
31	27.5		
32	36.5		
33	41.0		
34	34.0		
35	31.0		
36	37.0		
37	36.0		
38	47.0		
39	50.0		
40	57.0		

C. ANN AGE: 4 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	1.0	41	43.0
2	1.0	42	16.0
3	1.0	43	1.0
10	5.5	44	1.0
17	15.0	45	1.0
18	15.5	46	1.0
19	24.0	47	<1.0
20	23.0	48	<1.0*
21	28.0	49	3.1
22	13.0	50	14.0
23	12.0	51	14.0
24	6.7	52	20.5
25	4.4	53	31.0
26	5.6	54	15.0
27	3.3	55	19.5
28	2.4*	56	25.0
29	11.0	57	20.0
30	13.0	58	32.0
31	12.5	59	28.0
32	14.5	60	29.5
33	14.0		
34	24.0		
35	29.0		
36	38.0		
37	35.0		
38	44.0		
39	41.0		
40	66.0		

S. QUEENIE AGE: 4 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	5.9	41	36.5
2	4.1	42	33.0
3	3.7	43	48.0
10	9.3	44	40.0
17	9.0	45	40.5
18	7.7	46	38.0
19	5.2	47	42.0
20	3.6	48	38.0
21	5.0	49	10.5
22	5.0	50	2.8
23	3.7	51	2.3*
24	8.3	52	2.4
25	8.8	53	3.5
26	11.0	54	4.9
27	6.3	55	5.7
28	5.9	56	7.6
29	6.3	57	11.5
30	<1.0*	58	12.5
31	5.3	59	15.5
32	12.0	60	31.0
33	10.0		
34	9.0		
35	8.5		
36	12.5		
37	20.5		
38	16.5		
39	25.0		
40	34.5		

F. LOIS AGE: 4 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	5.0	41	7.1
2	5.2	42	4.6
3	6.1	43	4.5
10	6.0	44	3.7
17	6.0	45	4.0
18	6.0	46	1.8*
19	10.5	47	2.9
20	10.5	48	15.0
21	10.0	49	17.5
22	8.5	50	33.0
23	7.5	51	15.5
24	-	52	30.0
25	-	53	28.0
26	6.1		
27	6.1		
28	4.6		
29	1.0*		
30	10.0		
31	11.0		
32	7.6		
33	7.4		
34	10.5		
35	7.6		
36	9.8		
37	12.0		
38	30.0		
39	20.0		
40	15.5		

S. KATHY AGE: 4 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesteron Concn. (ng/ml)
1	1.7	41	42.0
2	8.8	42	48.0
3	8.8	43	42.0
10	12.0	44	16.5
17	40.0	45	17.5
18	47.5	46	19.0
19	51.0	47	16.0
20	44.0	48	13.0
21	48.0	49	18.0
22	53.0	50	2.3
23	54.0	51	1.0*
24	57.0		
25	60.0		
26	49.0		
27	7.5		
28	3.4		
29	1.4*		
30	2.6		
31	2.6		
32	1.5		
33	2.8		
34	12.5		
35	13.5		
36	18.0		
37	35.5		
38	40.0		
39	49.0		
40	36.0		

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Y. PRIMROSE AGE: 4 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesteron Concn. (ng/ml)
1	8.8	41	16.5
2	5.8	42	15.0
3	4.6	43	7.4
10	10.5	44	1.0
17	2.1	45	1.0*
18	1.7	46	1.0
19	1.2	47	5.9
20	1.0	48	5.7
21	1.0*	49	8.1
22	4.2	50	8.1
23	2.0		
24	1.5		
25	4.2		
26	6.0		
27	1.5		
28	6.0		
29	17.0		
30	27.0		
31	45.5		
32	37.0		
33	60.0		
34	55.0		
35	55.0		
36	38.0		
37	14.0		
38	17.0		
39	19.0		
40	17.5		

B. VENDOME AGE: 5 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	1.8	41	2.6
2	1.7	42	12.5
3	2.1	43	13.0
10	2.4	44	14.0
17	7.1	45	19.5
18	5.1	46	17.0
19	7.6	47	33.0
20	7.6	48	35.0
21	10.5	49	30.0
22	10.5	50	32.5
23	8.1	51	34.0
24	6.8	52	37.0
25	7.5	53	34.0
26	7.7	54	32.5
27	7.5	55	39.0
28	1.0	56	8.9
29	1.0	57	1.0*
30	1.0	58	1.2
31	<1.0	59	1.2
32	<1.0		
33	<1.0*		
34	1.0		
35	6.0		
36	3.4		
37	4.5		
38	2.8		
39	2.8		
40	2.6		

M. PRIMROSE AGE: 6 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progsterone Concn. (ng/ml)
1	1.9	41	18.0
2	1.2	42	10.0
3	1.1	43	12.0
10	5.9	44	9.5
17	9.6	45	8.2
18	1.0	46	7.4
19	8.4	47	6.7
20	9.6	48	7.0
21	1.0	49	7.5
22	1.0	50	6.2
23	1.0	51	9.3
24	1.0	52	11.0
25	1.3	53	12.0
26	1.2	54	10.5
27	<1.0*	55	12.0
28	6.2	56	8.8
29	7.3	57	7.3
30	-	58	6.0
31	-	59	7.5
32	6.8	60	12.0
33	6.8		
34	5.0		
35	10.5		
36	10.5		
37	6.5		
38	7.0		
39	5.6		
40	9.7		

R. FUSION AGE: 6 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	6.5	41	16.5
2	7.0	42	19.5
3	11.5	43	26.5
11	2.1	44	35.0
17	5.1		
18	4.0		
19	1.5*		
20	4.0		
21	3.0		
22	2.8		
23	2.6		
24	4.2		
25	2.5		
26	11.0		
27	17.0		
28	15.0		
29	17.5		
30	10.0		
31	1.0		
32	1.0		
33	<1.0*		
34	1.2		
35	1.2		
36	3.1		
37	3.3		
38	12.5		
39	12.5		
40	15.0		

D. PRIMETTE AGE: 6 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	7.5
2	7.8
3	6.2
10	1.8
17	1.7
18	4.9
19	5.2
20	8.9
21	4.3
22	9.7
23	4.2
24	4.6
25	1.0
26	1.0
27	1.0
28	1.0
29	1.3
30	5.4
31	4.8
32	2.5
33	1.0
34	2.5
35	2.5
36	<1.0*
37	1.5
38	2.1

T. NORA AGE: 8 Yrs.

Day Postpartum	Milk Progesterone Concn. (ng/ml)	Day Postpartum	Milk Progesterone Concn. (ng/ml)
1	-	42	14.0
2	6.5	43	14.5
3	5.7	44	14.0
11	5.9	45	9.3
18	8.0	46	11.5
19	7.1	47	12.5
20	7.5	48	15.5
21	7.5	49	17.5
22	6.0	50	15.5
23	5.2	51	1.9*
24	6.4	52	6.2
25	12.0	53	5.5
26	1.0	54	9.4
27	1.0		
28	2.6		
29	1.0		
30	1.0		
31	<1.0*		
32	5.8		
33	1.5		
34	1.2		
35	1.1		
36	1.1		
37	3.8		
38	6.3		
39	11.0		
40	11.0		
41	19.0		

BIBLIOGRAPHY

Abraham, A.M., R. Swerdloff, D. Tulchinsky, and W.D. Odell. 1971. Radioimmunoassay of plasma progesterone. Endocr. 32: 612.

Aono, T., A. Miyake, T. Shioji, T. Kinugasa, T. Onishi and K. Kurachi. 1976. Impaired LH release following exogenous estrogen administration in patients with amenorrhea - galactorrhea syndrome J. Clin. Endo. Meta. 42:696.

Appleyard, W.T., B. Cook, 1976. The detection of oestrous in dairy cattle. Vet. Rec. 99:253.

Araujo, P.G., G.N. Pizelli, M.R. de Carvalko and C.A. Menegnelli. 1974. Postpartum uterine involution and ovarian activity in cross-bred dairy cattle. Presg. Agropec. Boras. Ser. Vet. 9:1.

Arije, G.R., J.N. Wiltbank and M.L. Hopwood. 1974. Hormone levels in pre- and postparturient beef cows. J. Anim. Sci. 39:338.

Bozworth, R.W., G. Ward, E.P. Call, and E.R. Bonewitz. 1972. Analysis of factors affecting calving intervals of dairy cows. J. Dairy Sci., 55:334.

Britt, J.H. 1975. Early postpartum breeding in dairy cows. A review. J. Dairy Sci. 58:266.

Britt, J.H., R.J. Kittok and D.S. Harrison. 1974. Ovulation, estrus and endocrine response after GnRH in early postpartum cows. J. Anim. Sci. 39:915.

Buch, N.C. 1957. Reproduction studies in dairy cattle: I: Postpartum estrus and involution of the uterus. II: Survival of spermatozoa after freezing and thawing Diss. Abstr., 17:1173.

Buch, N.C., W.J. Tyler, and L.E. Casida. 1955. Postpartum estrus and involution of the uterus in an experimental herd of Holstein-Friesian cows. J. Dairy Sci., 38:73.

Bulman, D.C. 1978. Progesterone levels and fertility of lactating cows. Ph.D. thesis, University of Nottingham.

Carman, G.M. 1955. Interrelations of milk production and breeding efficiency in dairy cows. J. Anim. Sci., 14:753.

Carruthers, T.D., M. Kosugiyama and H.D. Hafs. 1977. Effects of suckling on interval to first postpartum ovulation and on serum luteinizing hormone and prolactin in Holsteins. Proc. A.S.A.S., p. 142.

Casida, L.E. 1971. The postpartum interval and its relation to fertility in the cow, sow and ewe. J. Anim. Sci., 32, Supl. 1:66.

Casida,L.E., W.E. Graves, J.W. Lauderdale, J.W. Riesen, S. Saiduddin, E.R. Hauser and W.J. Tyler. 1968. Studies on the postpartum cow. Res. Bulletin 270, University of Wisconsin, Madison.

Charnetzki, T.C., D.M. Galton, R.W. Adkinson and D.E. Mahle. 1978. Relationship of reproductive efficiency with days to first insemination and subsequent insemination. Proc. ADSA/ASAS Meeting, Michigan State Univ.

Cheredkov, S.N. 1970. Postcalving changes in the sex organs of Black Pied cows. Trudy belorussk. naucho-issled. Vet. Inst., 8:165.

Christensen, D.S., M.L. Hopwood and J.N. Wiltbank. 1974. Levels of hormones in the serum of cycling beef cows. J. Anim. Sci., 38:577.

Connor, J.S., R.L. Tribble, T.L. Woodward, J.R. Beverly, A.M. Sorensen Jr., and J.L. Fleeger. 1974. Serum estrogens in suckled and non-suckled heifers. J. Anim. Sci. 39:203. (Abstr.).

Darling, J.A.B., R.W. Kelly, A.H. Laing and R.A. Harkness. 1972. The isolation and identification of progesterone obtained from cow's milk during pregnancy. J. Endocr. 54:347.

Darling, J.A.B., A.H. Laing and R.A. Harkness. 1974. A Survey of the steroids in cow's milk. J. Endocr. 62:291.

Dobson, H., and R.J. Fitzpatrick. 1976. Clinical application of the progesterone-in-milk test. Br. Vet. J. 132:538.

Dobson, H., S.E. Midmer and R.J. Fitzpatrick. 1975. Relationship between progesterone concentrations in milk and plasma during the bovine oestrous cycle. Vet. Rec. 96:222.

Donaldson, L.E., J.M. Bassett and G.D. Thorburn. 1970. Peripheral plasma progesterone concentration of cows during puberty, estrous cycles, pregnancy and lactation and the effects of under-nutrition or exogenous oxytocin on progesterone concentrations. J. Endocrinol. 48:599.

Edgerton, L.A. and H.D. Hafs. 1973. Serum luteinizing hormone, prolactin, glucocorticoid and progestin in dairy cows from calving to gestation. J. Dairy Sci. 56:451.

El-Fouly, M.A., E.A. Kotby and H.E. El-Sobhy. 1976. Effect of suckling on uterine and cervical involution in postpartum Egyptian buffaloes. Indian J. Anim. Sci. 46:221.

England, B.G., E.R. Hauser, and L.E. Casida. 1973. Some effects of unilateral ovariectomy in the postpartum beef cow. J. Anim. Sci., 36:45.

Erb, R.E., V.L. Estergreen, Jr., W.R. Gomes, E.D. Plotka and O.L. Frost. 1968a. Progestin levels in corpora lutea and progesterone in ovarian venous and jugular vein blood plasma of the pregnant bovine. J. Dairy Sci. 51:401.

Erb, R.E., V.L. Estergreen, Jr., W.R. Gomes, E.D. Plotka and O.L. Frost. 1968b. Progestin content of ovaries and the effect on assessment of luteal activity in the bovine. J. Dairy Sci. 51:411.

Erb, R.E., A.H. Surve, C.J. Callahan, R.D. Randel, and H.A. Gaverick. 1971. Reproductive Steroids in the bovine. VII. Changes postpartum. J. Anim. Sci. 33:1060.

Fernandes, L.C., W.W. Thatcher, C.L. Wilcox and E.P. Call. 1978. LH release in response to GnRH during the postpartum period of dairy cows. J. Anim. Sci. 46:443.

Foote, R.H. 1978. Reproductive performance and problems in New York dairy herds. Search Agriculture 8:1. Cornell University Agric. Expt. Station Publication.

Gartland, P., J. Schiavo, C.E. Hall, R.H. Foote, and N.R. Scott. 1975. Detection of estrus in dairy cows by electrical measurements of vaginal mucus and by milk progesterone. J. Dairy Sci. 59:982.

Garverick, H.A., G.D. Niswender and C.J. Callahan. 1971. Reproductive steroids in the bovine. III. Changes during the estrous cycle. J. Anim. Sci. 32:946.

Gier, H.T. and G.B. Marion. 1968. Uterus of the cow after parturition: involutional changes. Amer. J. Vet. Res. 29:83.

Ginther, O.J., L.C. Nuti, M.C. Garcia, B.C. Wentworth and W.J. Tyler. 1976. Factors affecting progesterone concentration in cow's milk and dairy products. J. Anim. Sci. 42:155.

Gospodinov, G. and L. Kostov. 1976. Clinical and endocrinological studies on the puerperium in cows. Veterinarnomeditsinski Nauki 13:93.

Graves, W.E., J.W. Lauderdale, E.R. Hauser and L.E. Casida. 1968. Relation of postpartum interval to pituitary gonadotroplins, ovarian follicular development and fertility in beef cows. Res. Bull. 270. College of Agric. and Life Science, Univ. of Wisconsin, 23.

Harrison, D.S., C.E. Meadows, L.J. Boyd and J.H. Britt. 1974. Effect of interval to first service on reproduction, lactation, and culling in dairy cows. J. Dairy Sci. 51: 628 (Abstr.).

Heap, R.B., C.A. Bedford and J.L. Linzell. 1975a. Metabolic clearance rate, production rate and mammary uptake of progesterone in the goat. J. Endocrinol. 65:485.

Heap, R.B., M. Gwyn, J.A. Laing, and D.E. Walters. 1973. Pregnancy diagnosis in cows: changes in milk progesterone concentration during oestrous cycle and pregnancy as measured by a rapid radioimmunoassay. J. Agric. Sci., 81:151.

Heap, R.B., A. Henville and J.L. Linzell. 1975b. Metabolic clearance rate, production rate, and mammary uptake and metabolism of progesterone in cows. J. Endocrinol. 66:239.

Heap, R.B., R.J. Holdsworth, J.E. Gadsby, J.A. Laing, and D.E. Walters. 1976. Pregnancy Diagnosis in the cow from milk progesterone concentration. Br. Vet. J. 132:445.

Heap, R.B. and J.A. Laing. 1974. Pregnancy diagnosis in cows; progress report on the use of progestagen concentration in milk. British Cattle Breeders Club Digest #29.

Hendricks, D.M., J.F. Dickey, J.R. Hill and and W.E. Johnson. 1972. plasma estrogen and progesterone levels after mating and during late pregnancy and postpartum in cows. Endocrinol. 90:1336.

Hendricks, D.M., J.F. Dickey, G.D. Niswender. 1970. Serum Luteinizing hormone and plasma progesterone levels during the estrous cycle and early pregnancy in cows. Biology of reproduction 2:346.

Hoffmann, B., O. Gunzler, R. Hamburger and W. Schmidt. 1976. Milk progesterone as a parameter for fertility control in cattle; methodological approaches and present status of application in Germany. Br. Vet. J. 132:469.

Hoffmann, B. and R. Hamburger. 1973. Progesterone in der Milch: Radioimmunologische bestimmung, beziehunger zur Gelbkorperfunktron und Milchfettkonzentration. Zuchthyg. 8:154.

Hoffmann, B., R. Hamburger, O. Gunzler, L. Korndorfer, and H. Lohoff. 1974. Determination of progesterone in milk applied for pregnancy diagnosis in the cow. Theriogenology 2:21.

Howland, B.E., R.L. Kirkpatrick, A.L. Pope and L.E. Casida. 1966. Pituitary and ovarian function in ewes fed on two nutritional levels. J. Anim. Sci. 25:716.

Humphrey, W.D., D.R. Koritnik, C.C. Kaltenbach, T.G. Dunn and G.D. Niswender. 1976. Progesterone and LH in postpartum suckled beef cows. J. Anim. Sci. 43:290 (Abstr.).

Hunter, D.L., R.E. Erb, R.D. Randel, H.A. Garverick, C.J. Callahan and R.B. Harrington. 1970. Reproductive Steroids in the bovine. I. Relationships during late gestation. J. Anim. Sci. 30:47.

Johnson, A.D., J.E. Legates and L.C. Ulberg. 1966. Relationship between follicular cysts and milk production in dairy cattle. J. Dairy Sci. 49:865.

Kesler, D.J., H.A. Garverick, R.S. Yougquist, R.G. Elmore and C.J. Bierschwal. 1977. Effect of days postpartum and endogenous reproductive hormones on GnRH - induced LH release in dairy cows. J. Anim. Sci. 45:797.

Kohli, M.L., and K.R. Suri. 1957. The relation of some factors to the occurrence of postpartum oestrus in Hariana cattle. Indian J. Vet. Sci., 27:95.

Labhsetwar, S.P., W.E. Collins, W.J. Tyler and L.E. Casida. 1964. Some pituitary-ovarian relationships in the periparturient cow. J. Reprod. fertil. 8:85.

Laing, J.A. 1976. Progesterone assays of milk and the control of infertility. Br. Vet. J. 132:534.

Laing, J.A., and R.B. Heap. 1971. The concentration of progesterone in the milk of cows during the reproductive cycle. Br. Vet. J. 127:XIX.

Lamming, G.E. 1978. Reproduction during lactation; In: Control of ovulation. Eds: B.B. Crighton, G.R. Foxcroft, N.B. Haynes and G.E. Lamming. Butterworths, London.

Lamming, G.E., and D.C. Bulman. 1976. The use of milk progesterone radioimmunoassay in the diagnosis and treatment of subfertility in dairy cows. Br. Vet. J. 132-507.

Lamming, G.E., S.R. Moseley and J.R. McNeilly. 1974. Prolactin release in sheep. J. Reprod. Fertil. 40:157.

Lauderdale, J.W., W.E. Graves, E.R. Hauser and L.E. Casida. 1968. Relation of postpartum interval to corpus luteum development, pituitary prolactin activity, and uterine involution in beef cows. Wisc. Agr. Exp. Sta. Res. Bull. No. 270:42.

Louca, A. and J.E. Legates. 1968. Reproduction losses in dairy cattle due to days open. J. Dairy Sci. 51:573.

Lynn, J.E., S.H. McNutt and L.E. Casida. 1966. Effect of intrauterine bacterial inoculation and suckling on the bovine corpus luteum and uterus. Amer. J. Vet. Res. 27:1521.

Marion, G.B., and H.T. Gier. 1968. Factors affecting bovine ovarian activity after parturition. J. Anim. Sci. 27:1621.

Marion, G.B., J.S. Norwood and H.T. Gier. 1968. Uterus of the cow after parturition: factors affecting regression. Amer. J. Vet. Res. 29:71.

Matuszezak, R.I., J.S. Van Wie, R.R. Saatman, and R.H. Foote. 1974. Progesterone content of milk. J. Anim. Sci. 39:218.

Mauer, R.E., R.H. Rippel, M.T. Hedlund and R.E. Hutchinson. 1974. Plasma and milk progesterone, GnRH, and LH in cows. J. Anim. Sci. 39:218.

McClure, T.J. 1968b. Hypoglycaemia, an apparent cause of infertility of lactating cows. Brit. Vet. J. 124:3.

Moller, K. 1970. Uterine involution and ovarian activity after calving. New Zealand Vet. J. 18:140.

Morrow, D.A., S.J. Roberts and K. McEntee. 1969a. Postpartum ovarian activity and involution of the uterus and cervix in dairy cattle. I. Ovarian activity. Cornell Vet. 59:175.

Morrow, D.A., S.J. Roberts and K. McEntee. 1969b. A review of postpartum ovarian activity and involution of the uterus and cervix in cattle. Cornell Vet. 59 (No. 1): 134.

Morrow, D.A., J.J. Roberts, K. McEntee and H.G. Gray. 1966. Postpartum ovarian activity and uterine involution in dairy cattle. J. Am. Vet. Med. Assoc. 149:1596.

Moskalenko, N.I. 1970. The effect of season of the year on reproductive function in cattle. Trudy dal'nevost. nauchnoissled. Inst. sel'. Khox., No. 10, Pt. 3:49.

Norwood, J.W. 1963. Factors affecting postpartum regression of the bovine uterus. Ph.D. thesis, Kansas State Univ. Manhattan.

Nuti, L.C., B.C. Wentworth, H.J. Karavolas, W.J. Tyler and O.J. Ginther. 1975. Comparison of radioimmunoassay and gas-liquid chromatography analyses of progesterone concentrations in cow's milk. Proc. Soc. Exp. Bio. Med. 149:877.

Olds, D., and T. Cooper. 1970. Effect of postpartum rest period in dairy cattle on the occurrence of breeding abnormalities and on calving intervals. J. Am. Vet. Med. Assoc., 157:92.

Olds, D., and D.M. Seath. 1953. Repeatability, heritability and the effect of level of milk production on the occurrence of first estrus after calving in dairy cattle. J. Anim. Sci., 12:10.

Oxenreider, S.L. 1968. Effects of suckling and ovarian function on postpartum reproductive activity in beef cows. Am. J. Vet. Res., 29:2099.

Oxenreider, S.L., and W.C. Wagner. 1971. Effect of lactation and energy intake on postpartum ovarian activity in the cow. J. Anim. Sci. 33:1026.

Pennington, J.A., S.L. Spahr and J.R. Lodge. 1976. Factors affecting progesterone in milk for pregnancy diagnosis in dairy cattle. Br. Vet. J. 132:487.

Pennington, J.A., S.L. Spahr and J.R. Lodge. 1976a. Milk progesterone during the cow's reproductive cycle. J. Anim. Sci. 42:1361. (Abstr.).

Pennington, J.A., S.L. Spahr and J.R. Lodge. 1976b. Pregnancy diagnosis with unextracted cow's milk. J. Anim. Sci. 42:1361. (Abstr.).

Pope, G.S., S.K. Gupta, and I.B. Munro. 1969. Progesterone levels in the systemic plasma of pregnant, cycling and ovariectomized cows. J. Reprod. Fertil. 20:369.

Pope, G.S., I. Majzlik, P.J.H. Ball and J.D. Leaver. 1976. Use of progesterone concentrations in plasma and milk in the diagnosis of pregnancy in domestic cattle. Br. Vet. J. 132:497.

Quevedo, M.M., S. Saiduddin, and W.D. Foote. 1965. Influence of estradiol on reproductive activity in postpartum dairy cows. J. Anim. Sci. 24:587 (Abstr.).

Rakha, A.M., G. Igboeli, and J.L. King. 1971. Calving interval, gestation and postpartum periods of indigenous central African cattle under a restricted system of breeding. J. Anim. Sci. 32:507.

Randel, R.D. and R.E. Erb. 1971. Reproductive steroids in the bovine. VI. Changes and interrelationships from 0 to 260 days of pregnancy. J. Anim. Sci. 33:115.

Rawlings, N.C., L. Weir, B. Todd, J. Manns, J. Hyland, and D. Reiger. 1979. Some endocrine changes associated with the postpartum period of the cow. J. Anim. Sci., 49:Suppl. 1.

Riesen, J.W., S. Saiduddin, W.J. Tyler and L.E. Casida. 1968. Relation of postpartum interval to corpus luteun development, pituitary prolactin activity and uterine involution in dairy cows (effect of suckling). Wisc. Agr. Exp. Sta. Res. Bull. No. 270:27.

Roberts, S.J. 1955. Clinical observations on cystic ovaries in dairy cattle. Cornell Vet. 45:497.

Robertson, H.A. 1972. Sequential changes in plasma progesterone in the cow during the estrous cycle, pregnancy, at paturition and postpartum. Can. J. Anim. Sci. 52:645.

Rodbard, D. 1971. Statistical aspects of radioimmunoassays. In: Principles of Competitive Protein-Binding Assays. Eds: W.D. Odell and W.H. Daughaday. J.B. Lippincott Co., Philadelphia and Toronto.

Saiduddin, S. and W.D. Foote. 1964. Pituitary Luteinizing hormone activity of postpartum bovine. J. Amin. Sci. 23:592.

Saiduddin, S., J.W. Riesen, W.E. Graves, W.J. Tyler, and L.E. Casida. 1968a. Pituitary FSH activity in the postpartum dairy cow. J. Anim. Sci. 25:950.

Saiduddin, S., J.W. Riesen, W.E. Graves, W.J. Tyler, and L.E. Casida. 1968b. Effect of suckling on the interval from parturition to first estrus in dairy cows. J. Anim. Sci. 25:950.

Schiavo, J.J., R.L. Matuszczak, E.B. Oltenacu, and R.H. Foote. 1975. Milk progesterone in postpartum and pregnant cows as a monitor of reproductive status. J. Dairy Sci. 58:1713.

Short, R.E., R.A. Bellows, E.L. Moody, and B.E. Howland. 1972. Effects of suckling and mastectomy on bovine postpartum reproduction, J. Anim. Sci., 34:70.

Smith, V.R. 1959. "Physiology of Lactation", p. 176. Iowa State University Press, Ames.

Smith, V.G., L.A. Edgerton, H.D. Hafs, and E.M. Convey. 1973. Bovine serum estrogens, progestins and glucorticoids during late pregnancy, parturition and early lactation. J. Anim. Sci. 36:391.

Snedecor, G.W., and W.G. Cochran. 1967. Statistical methods. Iowa State University Press, Ames.

Snook, R.B., R.R. Saatman, and W. Hansel. 1971. Serum progesterone and luteinizing hormone levels during the bovine estrous cycle. Endocrinology 88:678.

Speicher, J.A., and C.E. Meadows. 1967. Milk production and costs associated with length of calving interval of Holstein cows. J. Dairy Sci. 50:975 (Abstr.).

Stabenfeldt, G.H., L.L. Ewing and L.E. McDonald. 1969. Peripheral plasma progesterone levels during the bovine estrous cycle. J. Reprod. Fert. 19:433.

Stevenson, J.S. and J.H. Britt. 1979. Relationships among luteinizing hormone, estradiol, progesterone, glucocorticoids, milk yield, body weight and postpartum ovarian activity in Holstein cows. J. Anim. Sci. 48:570.

Swanson, L.V., H.D. Hafs and D.A. Morrow. 1972. Ovarian characteristics and serum LH, prolactin, progesterone and glucocorticoid from first estrus to breeding size in Holstein heifers. J. Anim. Sci. 34:284.

Thatcher, W.W. and C.J. Wilcox. 1973. Postpartum estrus as an indicator of reproductive status in the dairy cow. J. Dairy Sci. 56:608.

Thibier, M. 1974. Elevage Insem. 144:27.

Thibier, M., C. Craplet, and M. Parex. 1973. Recl. Med. Vet. Ec. Alfort, 149:1181.

Thibier, M., J.F. Fourbet and M. Parex. 1976. Relationship between milk progesterone concentration and milk yield, fat and total nitrogen content. Br. Vet. J. 132:477.

Thorner, M.O., A.S. McNeilly, C. Hagan and G.M. Besser. 1974. Long-term treatment of galactorrhea and hypogonadism with bromocriptine. British Med. J. 2:419.

Touchberry, R.W., K. Rottensten, and H. Andersen. 1959. Associations between service interval, interval from first service to conception, number of services per conception, and level of butterfat production, J. Dairy Sci. 42:1157.

Tribble, R.L. 1973. Diss. Abstr. 34B:1251. As cited by Lamming, G.E. (1978) In: Control of ovulation, p. 335.

Tribble, R.L., A.M. Sorensen, P.L. Woodward, J.S. Connor, J.R. Beverly, and J.L. Fleeger. 1973. Serum progestins and luteinizing hormone levels in non-suckled primiparous heifers. Nature 246:494.

Wagner, W.C. 1970. Plasma corticoids in the cow. J. Anim. Sci. 31:233 (Abstr.).

Wagner, W.C. and W. Hansel. 1969. Reproductive physiology of the postpartum cow. I. Clinical and histological findings. J. Reprod. Fert. 18:493.

Wagner, W.C., R. Saatman and W. Hansel. 1969. Reproductive physiology of the postpartum cow. II. Pituitary, adrenal and thyroid function. J. Reprod. Fertil. 18:501.

Warnick, A.C., 1955. Factors associated with the interval from parturition to first estrus in beef cattle. J. Anim. Sci., 14:1003.

Webb, R., G.E. Lamming, N.B. Haynes, H.D. Hafs, and J.G. Manns. 1977. Response of cyclic and postpartum suckled cows to injections of synthetic LH-RH. J. Reprod. Fertil. 50:203.

Whitmore, H.L., W.J. Tyler and L.E. Casida. 1974. Effects of early postpartum breeding in dairy cattle. J. Anim. Sci. 38:339.

Wiltbank, J.N., and A.C. Cook. 1958. The comparative reproductive performance of nursed cows and milked cows. J. Anim. Sci. 17:640.

Wiltbank, J.N., W.W. Rowden, J.E. Ingalls and D.R. Zimmerman. 1965. Influence of postpartum energy level on reproductive performance of Hereford cows restricted in energy intake prior to calving. J. Anim. Sci. 23:1049.

Yuthasastrakosol, P. 1975. Hormone levels in ewes. Ph.D. Thesis, University of Manitoba.

Zoaral, J., and M. Polasek. 1969. Effect of feeding technique, season, and milk production on estrous cycles and conception in cows. Zivoc. Vyroba, 14:181.