UNIVERSITI PUTRA MALAYSIA

POSTPARTUM OVARIAN ACTIVITY IN SAHIWAL- FRIESIAN COWS FED DIFFERENT LEVELS OF ENERGY

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by

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ABSTRACT

Postpartum ovarian function was determined by milk progesterone radioimmunoassay in 21 first-calf Sahiwal-Friesian cows assigned to one of three treatment groups designated as low, medium and high energy levels (n=7). The progesterone concentrations in defatted milk were determined thrice weekly for 120 days postpartum.

Mean interval to resumption of ovarian activity and to forst ovulation was longest in the low energy group and shortest in the high energy group. Using progesterone concentration of > 1 ng/ml as criteria for corpus luteum activity, first ovulation occurred at 37.7 ± 18.0, 26.6 ± 15.7 and 25.3 ± 8.4 days for low, medium and high energy groups, respectively. However, first estrus was observed at 28.0 ± 21.6, 32.7 ± 9.8 and 25.4 ± 6.0 days, respectively. The percentages of silent estrus were 13.6 percent, 29.6 percent and 14.3 percent for low, medium

and high energy groups, respectively, with a majority (46.7 percent) detected during the initial postpartum estrous cycle. Interval to first insemination was longest in the low energy group, being 68.6 ± 10.3, 62.6 ± 18.1 and 56.6 ± 8.1 days for low, medium and high energy groups, respectively. However, services per conception were 1.1, 1.7 and 3.3, respectively. Interval to conception was shortest in the low energy group, being 74.3 ± 14.9, 79.1 ± 17.3 and 88.3 ± 30.4 days for low, medium and high energy groups, respectively. The corresponding first service conception rates were 85.7 percent, 42.9 percent and 42.9 percent. Although a trend was detected, the differences were not statistically significant ($P > 0.05$) between the groups on the reproductive parameters analysed.

In summary, low energy intake may delay the resumption of postpartum ovarian activity and ovulation in Sahiwal-Friesian cows. Mean interval from calving to conception, however, was not significantly different amongst the three groups if breeding commenced after 60 days postpartum. A higher incidence of silent estrus was detected during the initial estrous cycles after calving. Lastly, milk progesterone radioimmunoassay is a useful tool for estrus confirmation, early pregnancy diagnosis and assessment of fertility in postpartum cows.

CHAPTER I

INTRODUCTION

The dairy industry in Malaysia is progressing very slowly at present. Indigenous cattle with low productivity coupled with the availability of cheap, imported milk have had adverse effects on the development of the dairy industry (Osman Din, 1980; Mahendranathan, 1982).

The total dairy cattle population in Peninsular Malaysia is estimated at about 98,000 heads, comprising 20.4% of total cattle population (Tan,1983). With this small base population Malaysia was only able to produce about 2.5% of the nation's milk requirement (Mustaffa-Babjee, 1980). However, the government has put forth a policy that by 1990, the dairy industry is to produce 20% of the nation's milk requirement. To achieve this goal, the government embarked on a development strategy for improving large ruminant productivity by massive importation of crossbred breeding animals to augment the present cattle population and upgrade the native stock (Osman Din, 1980).

Under the Fourth Malaysia Plan (1980-1985), the government has been importing about 10,000 to 15,000 heads of crossbred cattle per annum (Osman Din, 1980; Mahendranathan, 1982), including about 5,000 heads of Sahiwal-Friesian cattle.

Another strategy towards further development of the dairy industry is through the active participation of smallholder farmers. The importation of crossbred cattle which enabled the farmers to possess better quality animals has helped encouraged more people to participate in dairy farming and milk production. The training of some 2,000 farmers undertaken from 1980, principally on cattle keeping for milk production, and the establishment of milk collecting centres (MCC) have further augmented the national effort of encouraging active participation of smallholder farmers (Mahendranathan, 1982).

Since reproduction is an important and integral part of animal production, the future projection for production of dairy cattle in the country will not only depend on the importation of exotic stock, but will also be dependent greatly on optimal reproductive performance. Indeed, profitable dairying will depend to a great extent on the fertility of both local and imported cattle (Tan, 1983).

Reviewing factors affecting fertility in postpartum cows, Samuel (1977) noted that reproductive inefficiency is a major problem in Malaysia. His study indicated that maximizing reproductive management practices especially in herds artificially maintained would be beneficial in improving efficiecy of reproduction.

Improved reproductive efficiency depends on being able to mate or inseminate the female at the time of optimum fertility and preferably on a pre-determined date; to know that she is

pregnant in the shortest possible interval after breeding and be able to calculate their calving date, and lastly to detect a lowered or lowering of fertility to allow treatment or culling of unproductive breeding stock (Melrose, 1979).

The task of improving reproductive management could, therefore, be made easier by having access to reliable means of detecting estrus, a proven system for estrus and/or ovulation control, a technic for early pregnancy diagnosis readily applicable under field condition, and a fertility control program including access to clinical expertise to allow diagnosis and treatment of infertile animals (Melrose, 1979).

At present, the available tools for reproductive management and identification of infertile or problem cows include such traditional technics as visual observation of mounting activity, use of heat-mount detectors and vasectomised bulls with chinball marker, as well as rectal palpation to examine the physical state of the ovaries and uterus. Recently, the technic of radioimmunoassay has been developed which has wide application in dairy cattle reproduction including confirmation of estrus, early pregnancy diagnosis and assessment of types of reproductive failure.

For dairy farming to be economically feasible, two aspects of management must be considered together. These are production management and reproductive management with nutrition being an integral part to consider since it affects both production and reproduction.

A good reproductive management program should aim for an optimum calving interval of 365 days for maximum milk yield. To attain this goal, the first standing estrus must be detected by 40-50 days postpartum and first insemination must be done by 60-70 days postpartum. It also requires an estrus detection rate of 80% and an average conception rate of 60%. The early postpartum period is, therefore, a very important stage for proper dairy cow management since it has a major effect on reproductive efficiency. For example, nutrition is very important during the early postpartum period as the cow starts lactating and peak milk yield is being approached. Besides adequate energy to meet the need of lactation, extra energy intake at this stage is required also to encourage early resumption of reproductive activity (Foote, 1975). With early resumption of postpartum ovarian activity, the cows can be rebred early in order to achieve a good calving interval.

At present, the Sahiwal-Friesian cattle is being imported widely into Malaysia to augment the numbers of local livestock and upgrade the productivity. Assessment of the adaptability and productivity of these animals is being carried out, and this includes the collection and documentation of data on reproductive performance. Hence, one of the objectives of this study was to assess the reproductive performance of imported Sahiwal-Friesian cows by monitoring postpartum ovarian activity using milk progesterone radioimmunoassay (RIA). Since the genetic disposition for high milk yield will only materialise with optimal feed supply, the role of nutrition, especially energy level, in early resumption of postpartum ovarian activity and

reestablishment of pregnancy was also investigated. The third objective was to assess the feasibility of using milk progesterone RIA as an aid in reproductive management.

CHAPTER II

REVIEW OF LITERATURE

Poor estrus detection and poor nutrition are the most likely causes of poor reproductive efficiency (McGowan, 1981). Hawk (1979) also considered reproductive inefficiency as the most expensive and frustrating problem confronting dairy production. Milk progesterone assay (Hawk, 1979) revealed that most problem cows were cycling and that the major causes of long calving intervals were "missed heats" and insemination of cows when they were not in estrus. The latter accounted for approximately 20% of normal services, thus lowering the conception rate.

1. Milk Progesterone Radioimmunoassay

Radioligand method for routine determination of progesterone in milk was first suggested by Laing and Heap (1971). It is clear that progesterone crosses the mammary gland in readily detectable amounts. The levels in milk of non-pregnant and late pregnant cows were similar to those reported in plasma (Laing and Heap, 1971). This finding opened up a new era of research with potential application and wide acceptability since it is easier to sample milk than blood. Furthermore, taking blood sample subjects the animal to undue stress compared to milk sampling.

It is well established that the changing concentrations of progesterone in the systemic circulation of cattle are due to secretion from the corpus luteum providing a useful indicator of ovarian physiology (Pope et al., 1969; Robertson, 1972; Schams et al., 1972; Thibier et al., 1976). With the availability of radioimmunoassay (RIA) technic, progesterone assay has been used increasingly to monitor ovarian activity and have been widely applied in the assessment of reproductive status of cows (Heap et al., 1976; Hoffmann et al., 1976; Lamming and Bulman, 1976; Cox et al., 1978; Ball and Jackson, 1979; van de Wiel et al., 1979).

It has also been shown that progesterone concentration in milk was highly correlated with those in peripheral plasma, showing a similar pattern (Schiavo et al., 1975). Various methods of milk progesterone assay have been described, including those by Gadsby et al. (1974), Heap et al. (1976), Hoffmann et al. (1976), Pope et al. (1976a), Claus and Rattenberger (1979) and Holdsworth et al. (1979).

While Hoffmann et al. (1976) and Claus and Rattenberger (1979) worked with progesterone concentration in milk fat, Pope et al. (1976a) worked with both milk fat and defatted milk plus whole milk and plasma too. Although progesterone in milk can be extracted using petroleum ether (Thibier et al., 1976), Heap et al. (1976) showed that it is more practical to use the direct, rapid method to analyse progesterone concentration in whole milk. Improving on the direct-rapid method of Heap et al. (1976), Holdsworth et al. (1979) developed a suitable automatic

procedure for milk progesterone RIA on a large scale basis.

Factors such as stage of estrous cycle and gestation, breed of animal, method and time of milk sampling seem to affect progesterone concentration in milk (Pennington et al., 1981). However, progesterone values from milk samples collected, preserved and stored correctly will provide a high degree of reliable information as to the reproductive status of dairy herds. Thus, progesterone RIA technic is a valuable diagnostic tool in fertility control and reproductive management.

In a study by Thibier et al. (1976), a close correlation between progesterone content of peripheral plasma and milk during the estrous cycle was found. Hoffmann and Hamburger (1973) had shown also that there was a close correlation between levels in plasma and milk, especially for milk with three percent fat content.

According to Pope et al. (1976a), the relationship of progesterone in whole milk (y ng/ml) to that in plasma (x ng/ml) was $y = 1.94x + 0.27$ ($r = 0.91$). This means progesterone levels in whole milk is about twice that in plasma.

Since a close correlation between progesterone content of plasma and that of milk has been well-proven, many researchers opt for using milk sample in their investigation. This is because collection of milk samples eliminates undue stress associated with bleeding and milk sampling can be easily incorporated into the daily milking routine (Laing and Heap, 1971). Thus,

milk progesterone assays are now being used more extensively and routinely in surveys of ovarian function in herds of dairy cows (Pope and Swineburne, 1980).

Progesterone levels in milk, however, is more variable. Considerable variation in milk progesterone concentration was reported by Booth (1979) to be associated with the fat fraction of milk. Hoffmann and Hamburger (1973) noted that as a general rule, progesterone concentration in milk increased by 3 ng/ml for each percent unit increase in milk fat. Studying the partitioning of progesterone between the aqueous and fat phase of milk, Pope et al. (1976b) found in milk of four percent fat (mean value of bulk milk of British-Friesian cows), 13% of progesterone was in the aqueous phase while 87% was in the fat phase.

Variation in the fat level in bovine milk is considerable (Pope et al., 1976a,b; Dodd and Griffin, 1979). Fat content of milk may differ according to the physiological state of the cow (King, 1977), diet of the cow, the udder quarter from which it is drawn as well as how and when the milk sample is taken. The fat content also may differ markedly between milk of different breeds and between individuals within breeds (Pope and Swineburne, 1980). Since fat level is associated with variation in progesterone concentrations, this might in turn seriously influence the interpretation of physiological studies.

Pope et al. (1976a,b) found that levels of progesterone in whole milk collected at different times and stages of milking are dependent not only on levels of progesterone in plasma

at time of sampling, but also on the fat content of the sample, which is itself very dependent on the timing and method of sampling.

The reason why progesterone content in whole milk is dependent on how milk sample was taken during the milking process is because the fore-milk, which is hand drawn before the main milking process has a lower fat content than the composite milk (Pope and Swineburne, 1980). This difference in milk fat percent of samples taken at different stages of milking may be due to partial separation of milk phases within the udder.

Pope et al. (1976a) measuring fat and progesterone in fore-milk, composite milk and last-milk taken at evening and also following morning milking found a correlation of $r = 0.8$ between fat and progesterone levels. In agreement with Pope et al. (1976a), Pennington et al. (1981) also noted that method and time of sampling had a significant effect ($P < 0.01$) on progesterone concentration in milk. Progesterone content in fore-milk, composite milk and last-milk was associated with fat content. Correlation coefficient was 0.65 ($P < 0.01$) between fat percent and progesterone concentration when milk from all methods of sampling were included.

Ginther et al. (1976) measured concentrations of fat and progesterone in milk samples taken at different times of the day and found a correlation of $r = 0.98$ between fat and progesterone levels. Batra et al. (1980) found that in cows milked twice a day, progesterone concentrations in both whole milk and

in milk fat were higher in the evening samples. The higher levels of progesterone in evening milk could be due to the higher levels of fat in these samples (Dodd and Griffin, 1979; McCaughey and Gordon, 1979; Batra et al., 1980).

Pennington et al. (1981) agreed with the above researchers that the difference in progesterone level in morning and evening milk is probably caused by milk fat content of sample because they found that fat percent of morning milk is 1.7% lower than evening milk. The average evening milk fat content is about 3.6%. Therefore, there is evidently variation in the secretion rate of progesterone into milk associated with the time of day.

The relationship between varying levels of progesterone in the plasma of cows during different physiological states is closer to that in defatted milk or milk fat than in whole milk. Thus, when it is desirable to monitor physiological changes, it is best to measure progesterone in either milkfat or fat-free aqueous phase (Ball and Pope, 1976; Pope et al., 1976b; Pope and Swineburne, 1980; Oltner and Edqvist, 1981).

Although milk fat samples are better than whole milk samples because 88% of progesterone goes into the fat portion (Eastman, 1979), an increasing number of investigators are using defatted milk samples to eliminate entirely the variation due to milk fat content. When defatted for progesterone estimation, samples taken from any quarter of the udder, from different milking fractions or taken at any time of the day can be assayed without significantly reducing the accuracy of the assessment (Pope et al., 1976b; McCaughey and Gordon, 1979; Oltner and Edqvist, 1981).

Another advantage of using defatted milk is it avoids the necessity for relatively complicated separation and extraction procedure (Pope and Swineburne, 1980). Furthermore, assay of defatted milk requires less antibody since higher dilutions of antibody can be made, for example, 1:100,000 in contrast to whole milk assay, 1:12,500 (Pope et al., 1976a).

Booth(1980) observed some breed difference in milk progesterone content, and this he believed is connected to the fat percent. He found that Jersey cows with higher percent milk fat had higher progesterone levels than Friesian cows. Since this difference is believed to be due to fat content, Ball (1980) encouraged the use of defatted milk to eliminate this "breed" difference in progesterone level, especially for herd surveys.

To sum up, the use of defatted milk eliminates variation due to milk fat content, enabling milk samples to be collected at any time of the day, stage of the milking process and from any quarter of the udder. This means sample collection can readily be done without a strict standardized regime or close supervision. Furthermore, progesterone determination can be done on any breed without jeopardizing the accuracy, and it also facilitates the interpretation and comparision of results by various workers without the complication of having to relate the results to type of sample taken and breed used.

2. Reproduction in Dairy Cattle

The most notable feature of the adult female reproductive system is the total absence of a steady state. It is a dynamic

system with an ever changing functional status. Few organs change their gross appearance or even their functional form day to day like the cycling ovary. The ovary changes from a gametogenic function to an important endocrine function every 17 to 21 days in polyestrous domestic animals, such as the cow (McDonald, 1977).

Changes in the reproductive tract during the estrous cycle result from a complex physiological interaction involving the hypothalamus, anterior pituitary gland and the gonads (Bath et al., 1978).

a. Estrous Cycle

A rhythmic sexual behavior pattern develops in the female during puberty. This behavioral change is cyclic in nature. The combination of physiological events which begin at one estrous period and end at the next is termed an estrous cycle. The average length of the estrous cycle is about 20 days for heifers, and 21-22 days for mature cows with some individual variation. Although the mean length in the cow is 21.3 ± 3.7 days, 30% of all estrous cycles are < 17 or > 25 days in length. Thus, it can be seen that considerable variation can be expected under normal conditions (Frandson, 1974; McDonald, 1977).

Estrus is defined as the period of sexual receptivity. Estrus lasts for approximately 18 hrs for heifers and 20 hrs for cows (Frandson, 1974; McDonald, 1977).

Ovulation is an important event of the estrous cycle. It occurs about 10-15 hrs after standing heat. Immediately after ovulation, the corpus luteum begins organisation and development. The corpus luteum is a temporary endocrine organ which functions temporarily in the cycling non-pregnant animal, but throughout most of pregnancy in many animals. Its main function is the production of progesterone (McDonald, 1977).

If day 1 refers to day of estrus, a blood clot would have filled the ruptured, ovulated follicle by day 4. After ovulation, cells from the theca interna and particularly, the granulosa layer, are reorganised into functional luteal tissue interspersed by a rapid developing blood supply. Considerable progesterone production begins by day 5 and by day 6 the corpus luteum has become more distinct. Development of the corpus luteum is complete by day 7 with increasing progesterone secretion. During the second week, the physical changes are minimal but the output of progesterone is maximal (Frandson, 1974; McDonald, 1977).

Day 14 and 15 are critical to the life of the corpus luteum. If conception occurs, then the corpus luteum will continue to function by secreting progesterone to maintain pregnancy. If the uterus does not contain a zygote by day 15, then the corpus luteum will begin degeneration or luteolysis. With decreased vascularity and shrinkage of corpus luteum, progesterone production stops and the level