The Use of Milk Progesterone Assays for Reproductive Management

Dairy Integrated Reproductive Management

Dr. R.C. Rhodes, Ill
University of Rhode Island

Estrous detection and early pregnancy diagnosis are two common reproductive management problems dairy producers face. For the last decade, producers in Great Britain have been utilizing results from milk progesterone assays as a tool to minimize these problems. Producers in the United States have begun to adopt this technology. However, significantly less than 10% of all herds are currently using milk progesterone as a diagnostic aid.

Progesterone

The corpus luteum (CL) develops on the ovarian site where the follicle ruptured and the ovum (egg) was released around the time of estrus (heat, Fact Sheet IRM-2). The CL produces the hormone progesterone (P_4), which is needed for pregnancy maintenance. Progesterone produced by the CL is secreted into the blood and subsequently into milk.

The concentration of P_4 is higher in milk than blood; however, they are closely correlated. Therefore, P_4 concentration in milk is an accurate way to monitor ovarian activity, and, hence, reproductive status.

In a normally cycling cow, levels of P_4 are relatively low in both blood and milk around estrus. Then P_4 increases in both fluids. On day 17 or 18 of the estrous cycle, progesterone declines as a new follicle develops. If a cow is pregnant, P_4 levels would not decrease on day 17 or 18, but would remain elevated (Fig. 1A and 1 B).

Estrous Detection

Estrous detection (Fact Sheet IRM-6) is a problem that plagues producers. As many as 50% of all dairy producers do not have a planned program for estrous detection; therefore, they may not recognize the problem.

Even with a planned program, the task of estrous detection may be performed inadequately. Up to 20% of all cows are inseminated at an incorrect time, greatly reducing the success of insemination (Fact Sheet IRM-10). For example, in Fig. 2 cow 526-H was bred on day 96 postpartum, 10 days after she was in estrus based on milk P_4.

The use of milk P_4 to augment estrous detection can apply to open cows. Progesterone levels in the cow are an excellent indicator of ovarian activity. When a cow has a high concentration of milk P_4 she probably has a CL and is not in estrus. An example is the high P_4 concentration observed on day 50 in cow 468-H, Fig. 2.

If a cow has a low concentration of milk P_4 she may be in estrus, just out of estrus or not cycling. Cow 448-H (Fig. 2) has low P_4 values on day 30 due to a lack of ovarian activity. In contrast, low P_4 values observed in cow 464-H on day 45 were due to the fact that CL regressed (Fact Sheet IRM-2) and the cow was approaching estrus. In fact, the cow was bred on day 46.

Milk P_4 also can be used to detect missed estrus. For example, cow 464-H (Fig. 2) had low levels on day 70; however, no estrus was observed during this period.

The primary limitations of milk P_4 for estrous detection is the number of samples required to adequately provide a clear hormonal profile. Milk P_4 measurements cannot substitute for regular visual observation, but can serve as an estrous detection aid (Fact Sheet IRM-7).

Early Pregnancy Detection

The most common use of milk P_4 measurements is for early pregnancy detection. Typically, milk P_4 is determined 21 to 23 days after insemination. Although many cows that have not been bred and are not pregnant show signs of estrus, many do not. Whether the lack of estrus is due to a "cow" problem or "human" problem is unclear. Therefore, the use of milk P_4 measurements from cows 20-23 days after insemination that have not shown signs of estrus, would be beneficial. Cows that were pregnant would have a high milk P_4 level. For example, high P_4 values were observed in cow 468-H (Fig. 2) 21 days after breeding, indicating a successful insemination. Cows with low P_4 would not be pregnant and would be close to or a little past their normal time of estrus or not cycling. Thus, producers could identify cows that were not showing estrus and closely follow their reproductive performance.

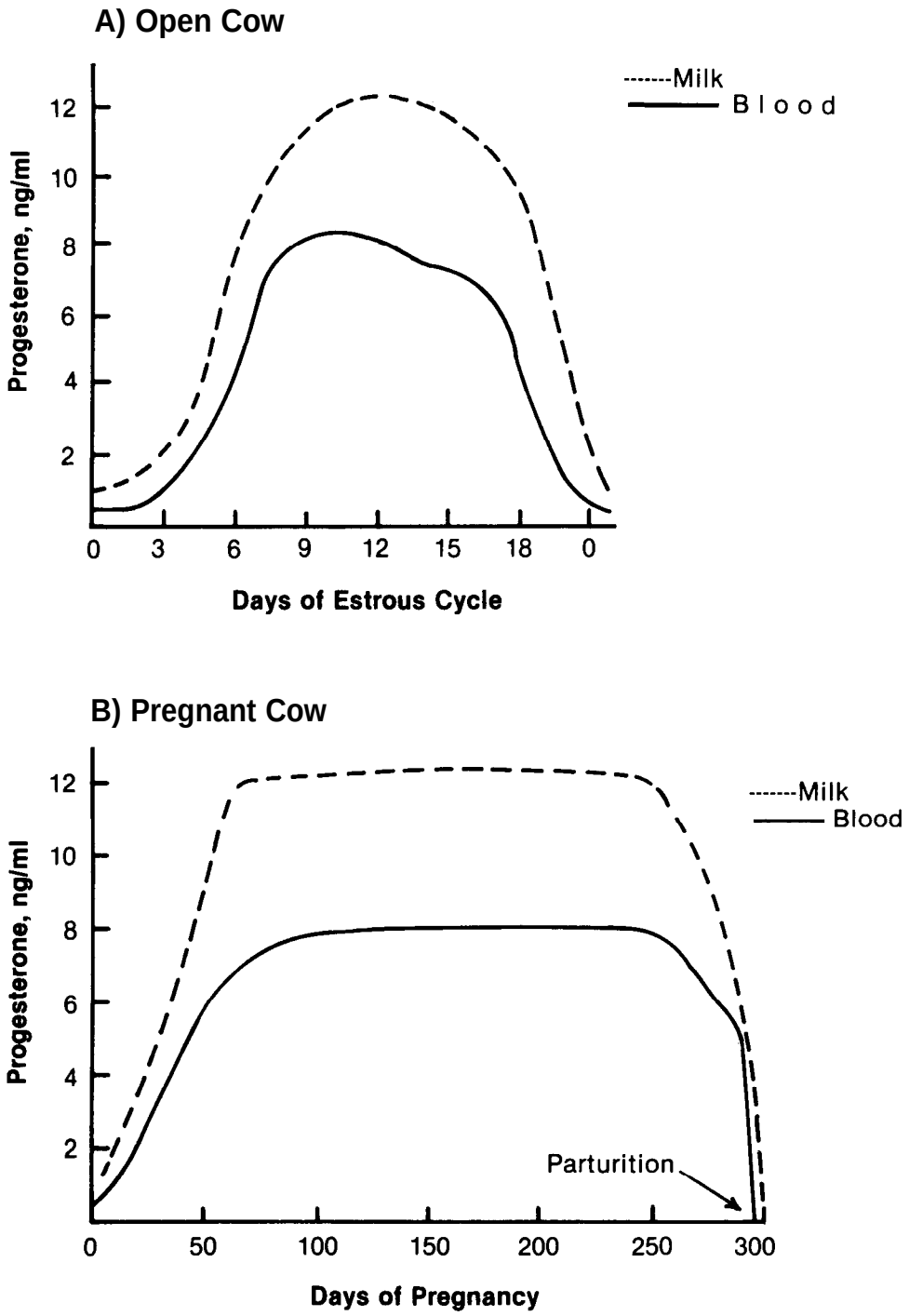

Fig. 1. Blood and milk progesterone concentrations in A) an open cow during one estrous cycle and B) a pregnant cow from insemination to parturition.

Milk P_4 might be used to identify true "problem" cows also. Subfertility can be due to a variety of causes including embryonic loss (cow 526-H, Fig.2; prolonged high P_4 followed by low P_4 levels), luteal cysts (cow 468-H, Fig. 2; prolonged high P_4 levels, no pregnancy; Fact Sheet IRM-25), metritis (medium to high P_4 levels, no pregnancy; Fact Sheet IRM-22) and anestrous (cow 448-H, Fig. 2; low P_4 levels; Fact Sheet IRM-7). Early detection of these problems helps avoid the financial loss (Fact Sheet IRM-17) associated with undiagnosed, untreated subfertile cows. Close communication between the veterinarian and producer in combination with knowledge of P_4 patterns can make milk P_4 levels a valuable diagnostic tool.

Procedure

The procedure used for obtaining a milk sample is relatively simple. Research has shown a sample of strippings following last milk, provide accurate P_4 concentrations. Proportionate samples from weigh jars or milk bucket machines can also be used. However, samples of foremilk do not give an adequate reading of P_4 due to a high variable of fat and, hence, P_4 content.

For pregnancy testing, the time of sampling is critical. Samples are obtained between 21 and 24 days after breeding. The primary advantage of sampling on day 23 or 24 is cows which did not conceive return in estrus by day 21 or 22. Only cows which did not show estrus are then sampled on day 23 or 24.

The use of P_4 in estrous detection is more time consuming and tedious than for pregnancy detection. Typically, estrus assessment using P_4 is primarily for the "problem" cow. These cows are sampled every 3 days and P_4 values recorded. The advantage of this type of sampling is ovarian activity of the cow can be characterized. The primary disadvantage is the cost of P_4 analysis and the labor involved in obtaining multiple samples. A second disadvantage is currently, samples can only be submitted by a veterinarian for milk progesterone analysis.

Sample bottles contain a chemical pill (potassium dichromate) to preserve the milk. This chemical does not effect the concentration of milk P_4. Once the samples have been collected in the vials, they should be refrigerated. Samples may accumulate over several days prior to shipping. The maximum storage period of the refrigerated, preserved sample prior to shipping, is about 10 days.

Once the results of the P_4 analysis are received, P_4 values should be carefully evaluated. Under normal circumstances, high P_4 values (greater than 3 ng/ml) indicate the cow is either pregnant or in the middle of her estrous cycle. Midrange values (1-3 ng/ml) indicate that pregnancy is questionable. Low values (less than 1 ng/ml) indicate the cow is not pregnant and that she is either close to estrus, in estrus or not cycling. Interestingly, the relative values of P_4 do not differ among dairy breeds and, hence, interpretation of a value is the same for a Jersey, Ayrshire or Holstein.

The accuracy of the test is high in both field and laboratory settings. Low P_4 results are 95-100% accurate in predicting cows that are not pregnant. Mid-range values are 93% effective in determining cows that are not pregnant. High P_4 levels are 80% accurate in determining cows that are pregnant.

However, producers should realize there is some margin of error associated with the test. Errors due to the cow could be associated with embryonic mortality, pyometra or an active case of mastitis. The diagnostic capabilities of a milk P_4 assay are disguised by mastitis. Milk from mastitic quarters gives a low P_4 value even though the cow might be pregnant.

Producer errors might be associated with poor estrous detection and breeding, incorrectly identifying an animal or sampling the wrong cow. However, with proper precautions most human errors can be avoided.

The milk P_4 assay system is not a cure-all. However, the system has marked advantages, including ease of obtaining samples, early pregnancy determination and aiding in diagnosing reproductive problems. Additionally, the herd veterinarian may find the test an aid in providing additional information about a specific cow.

However, there are limitations to this system. These included erroneous results, the critical period of sampling and, perhaps, the tendency to substitute milk P_4 analysis for routine veterinary care.

New Horizons

New procedures are currently being tested for more rapid analysis of milk P_4. Currently, most laboratories use a sensitive radioimmunoassay (RIA) to determine P_4 levels. The primary disadvantages of this system are cost and the need for very technologically advanced equipment.

Recently, several laboratories in the United States and Great Britain have developed and are testing new simplified analytical procedures called enzyme immunoassays (EIA). Washington State University researchers have shown EIA to give results similar to the RIA. The primary advantage of the EIA is that it can potentially be performed "on the farm" by a trained technician with same-day results. An EIA for P_4 has recently become commercially available from Noctech Limited, Dublin, Ireland.

The benefit of the milk P_4 test to the dairy industry is the ability to diagnose pregnant or open cows by 21-24 days after breeding. In the event the cow, which was inseminated 21 days earlier, is open, she can, potentially be rebred earlier or receive special attention. The ultimate benefit of milk P_4 is having more cows with optimum calving intervals.

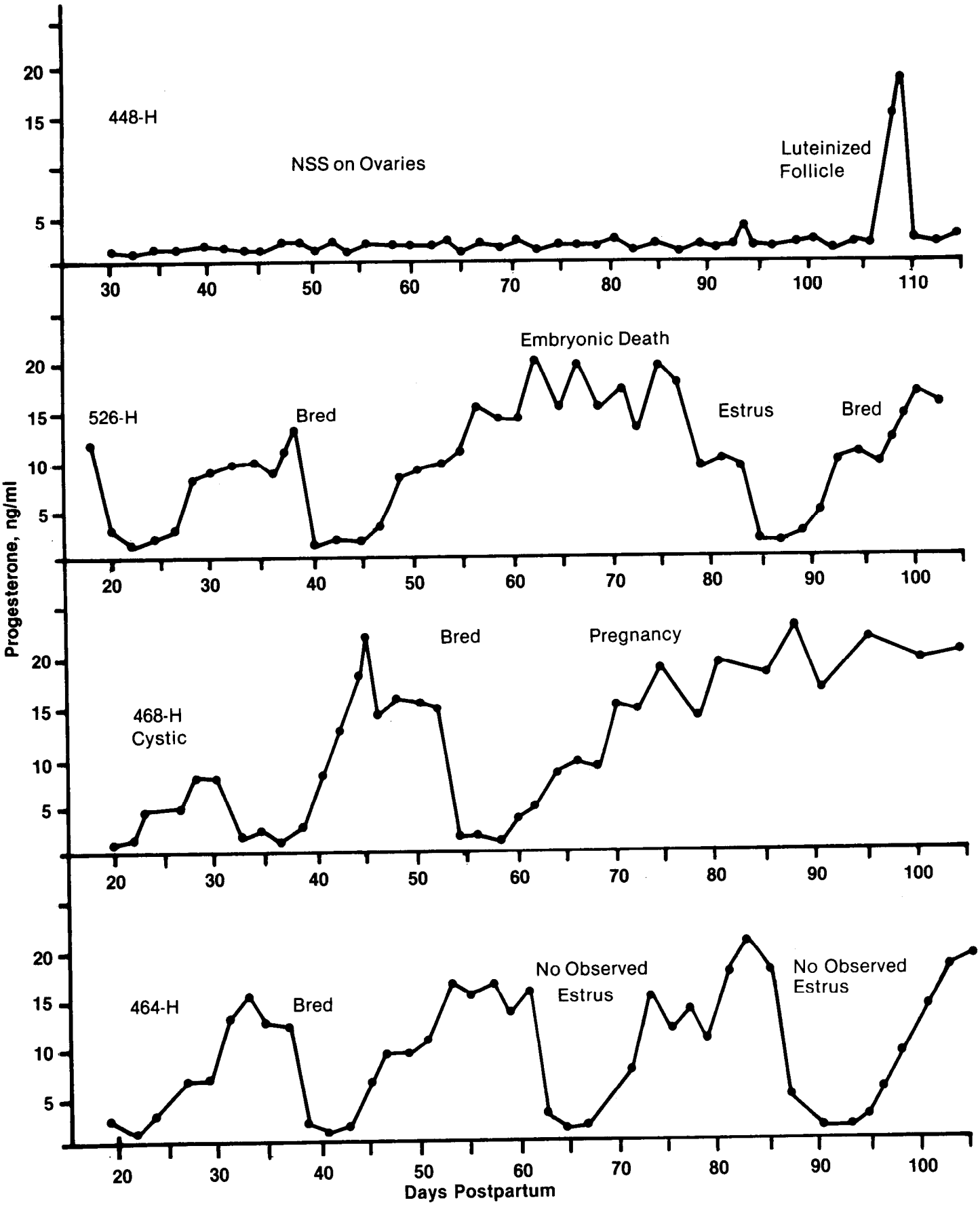

Fig. 2. Actual progesterone profiles of four cows.