



Milk progesterone analysis for determining reproductive status

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INTRODUCTION

Poor reproductive performance is one of the most costly and difficult problems for dairy and livestock producers. Even in some well-managed herds, reproductive failure continues to be one of the primary reasons why cows are culled. Depending on the level of milk production in the herd and variable costs associated with poor reproductive management, a dairy producer loses between \$1 to \$3 per cow each day the cow is open (not pregnant) beyond the 90 days post-calving.

Inaccurate or inefficient heat detection is still the major cause of low conception rates and long calving intervals. But there is a tool to help herd managers and veterinarians troubleshoot causes of poor reproductive performance--especially problems associated with heat detection. The tool is *milk progesterone analysis*.

Scientists have tested milk samples for many years to monitor progesterone levels in cattle. The assay they use is a very precise radioimmunoassay, requiring sophisticated equipment, radioactive tracers, and skilled technicians to perform the analysis. Radioimmunoassay for progesterone in

milk has been used on a commercial basis in Europe since 1973, with as many as 100,000 cows tested in 1980. But samples had to be submitted to a centralized laboratory for analysis, which was somewhat costly and time-consuming. Such testing was available from certain laboratories here in the United States.

In the mid-1980's, kits for performing the *progesterone enzyme immunoassay* procedure became commercially available to dairy producers and veterinarians. This procedure is considered a cow-side test since it can be performed on the farm or in a veterinary clinic. It is important, however that the farmer receives proper instruction on milk sampling, assay procedures, and interpretation of the results.

This enzyme immunoassay is designed to determine relative rather than absolute concentrations of progesterone, and results are classified as either low or high. In most kits, assays produce a color reaction that can be read visually or through an electronic scanner. Results, usually obtained within a half-hour, are compared with a standard containing known concentrations of progesterone.

PHYSIOLOGY

Progesterone is a hormone produced and released into the blood by the corpus luteum on the ovary. The corpus luteum is formed after the follicle has ovulated. This hormone is low during heat, and begins to rise after ovulation as the corpus luteum develops. If the cow was bred and becomes pregnant, progesterone in blood and milk remains high until just prior to calving. If the cow does not conceive, the corpus luteum begins to degenerate on approximately day 17 of the cycle, and progesterone declines to minimal concentrations on days 20 through 23 as the cow returns to heat.

The concentration of progesterone in the blood is correlated closely with the concentration in milk. In fact, since progesterone is a steroid hormone, it has an affinity for milk fat; thus progesterone in milk is somewhat higher than in the blood. However, the relative relationship

between milk and blood levels is the same. Variation in the concentration of progesterone in milk due to stage of the cycle or pregnancy status is much greater than the effect caused by the variation in fat content

Some kits require obtaining foremilk samples, while others require postmilk strippings. It is important to use the type of sample specified in the instructions, and follow instructions carefully. The number of steps involved in performing the test varies among kits. Milk should not be obtained from quarters that are infected (mastitis).

Samples can be obtained and refrigerated with a preservative (potassium dichromate) for 7 to 10 days before analysis. It is generally less expensive to run a batch of milk samples through the assay at one time than to assay single samples. In most cases, however, dairy producers want to know the results immediately.

USES OF MILK PROGESTERONE ANALYSIS

1. Verify suspicious heats. If the herdsperson is suspicious that a cow is in heat, progesterone concentrations can be used to verify if indeed the cow is in or near heat. Milk progesterone testing may be useful in verifying estrus if:
 - The cow was observed in heat, but was previously diagnosed pregnant.
 - Standing heat was observed, but the interestrus interval was abnormally long.
 - Cows were detected in heat based solely on secondary signs of heat.
2. Evaluate accuracy of heat detection or identify errors in estrous detection.
3. Identify open cows.
4. Monitor postpartum ovarian status.
5. Differentiate types of ovarian cysts.
6. Evaluate response to various hormonal treatments.

EVALUATION OF HEAT DETECTION ACCURACY

Before discussing the usefulness of milk progesterone in the evaluation of a heat detection program, it is important to appreciate some of the obvious symptoms of inefficient heat detection (missed heats) and inaccurate (errors) heat detection.

Herds with missed heats

- Few heats recorded before first service
- First service more than 80 days (average) after calving.
- Too many 38 to 45- and 55 to 65-day intervals between breedings.

Herds with heat detection errors

- More than 10 percent estrous cycles between 3 and 17 days
- More than 10 percent estrous cycles in the 25- to 35-day range
- More than 10 percent of cows bred one day, and again two to three days later
- Cows checked pregnant to a service earlier than the last service record
- Cows calving normally three to six weeks before expected to calve

A comprehensive study in 476 herds in several Northeastern states revealed a 5 percent error rate in heat detection on the day of breeding, and as high as 60 percent in some herds. The error in heat detection was 10 percent or greater in nearly one-third of the

herds. That is, cows were being bred when progesterone concentrations were high. Thus these cows were not in or near heat when inseminated. Milk progesterone testing can be an accurate method of evaluating heat detection on an individual basis.

To make the evaluation worthwhile, 15 to 20 cows should be sampled on the day of insemination. Milk samples should be obtained at the milking immediately after insemination. To save money, samples can be preserved and refrigerated to be analyzed as a batch. When compared with a standard progesterone sample, the milk samples obtained on a day of breeding should have low progesterone. The color reaction that indicates the relative level of progesterone will vary among the assay kits manufactured by different companies.

If more than 5 to 10 percent of the samples have high levels of progesterone, the heat detection error rate is too high. A few samples may have intermediate concentrations of progesterone suggesting that progesterone may be declining; but not yet have reached a minimal level; or that it is rising and low concentrations have already occurred. No definitive interpretation can be made from such results.

Dairy producers and veterinarians must realize that progesterone is low for several days around the time of heat. Thus low progesterone indicates the cow is either in or near heat, and progesterone levels cannot be used to precisely time the insemination. Errors in heat detection should be considered the primary cause of low conception in problem herds. Milk progesterone analysis is a tool to help verify if a heat detection problem exists.

DETERMINE PREGNANCY STATUS

Low progesterone levels at 19 to 24 days postbreeding are usually 95 percent accurate in identifying cows that are not pregnant. Obtaining milk samples 19 or 20 days postbreeding will alert the dairy producer to closely observe for heat those cows with low levels of progesterone. There is an advantage of reduced cost by waiting until 23 or 24 days after breeding, since only cows that have not returned estrus will need sampling. Most cows will return heat by 23 days.

However, several studies in the U.S. and Europe have evaluated milk progesterone analysis as a method of pregnancy diagnosis. Generally it has been shown that high progesterone 20 to 24 days postbreeding is 75 percent accurate in confirming pregnancy. It must be realized that conditions other than pregnancy can cause high progesterone concentrations 20 to 23 days postbreeding.

These include severe uterine infection, some cystic ovarian conditions, estrous cycles 24 to 28 days in length, and early embryonic death.

Day of sampling is critical. Sampling after day 24 is unacceptable because most open cows begin the upward trend of progesterone secretion that would cause a false positive pregnancy diagnosis. Furthermore, if the cow was initially bred when not in or near heat (luteal phase, high progesterone), the 20 to 24 day sample would indicate high progesterone when indeed she is not pregnant but in the normal luteal phase of the cycle.

This test should not replace routine veterinary reproductive examinations and pregnancy checks. Milk progesterone testing for pregnancy status would be useful in areas where the dairy cattle population is low, and a competent veterinarian may not be available for routine examinations.

MONITOR OVARIAN STATUS

Veterinarians and specialists who understand hormonal patterns associated with certain reproductive dysfunctions and the anticipated progesterone response to specific treatments for reproductive problems can use milk progesterone analysis as a diagnostic tool. Generally this involves collecting milk samples from problem cows at specified intervals to provide a progesterone profile of ovarian activity.

If milk samples are collected frequently at specified intervals, this tool can be used to detect anestrus

cows and monitor response of treatment with various treatments such as prostaglandins. It may also be helpful in differentiating between follicular and luteal cysts..

Since prostaglandins are being used more frequently in synchronization programs for lactating cattle--and the effectiveness of this treatment depends on the presence of a functional corpus luteum on the ovary--milk progesterone analysis can be useful in verifying if a corpus luteum is present.

TIPS FOR USING MILK PROGESTERONE TESTS

1. Users of progesterone assays must be cautious about the limitation of the technique. Some commercial literature exaggerate claims about the usefulness of the test, and frequently the interpretation of the results is too broad. This is especially true with regard to diagnosis of certain reproductive disorders.
2. The ease and convenience of obtaining milk samples as opposed to blood samples, and the fact that results of the assay can be seen visually by a color reaction, make this especially helpful for use at the farm level. It should be noted that

the milk sample must be mixed with other solutions and incubated for a specified time period. Directions must be followed carefully, or false readings will be obtained. These cow-side assays determine whether milk progesterone concentrations are low or high in comparison with standard progesterone levels. They are not designed to indicate absolute progesterone concentrations. Some kits can be used in a semi-quantitative manner when a spectrophotometer (light absorbency meter) and range of standards are used to plot a standard curve. These can be used in a clinic, but are not practical for farm use.

TIPS FOR USING TESTS (CONTINUED)

3. It is important that dairy producers who use milk progesterone kits understand the hormonal changes that occur during the estrous cycle of the cow. They also must realize the importance of timely sampling and correct sampling technique.
4. Dairy producers interested in trying this new technology should buy a kit with a small number of tests per package to become familiar with the procedures and determine its practicality before investing too much money. The number of tests/kit will vary from 10 to 96. The cost per test ranges from \$3 to \$5, depending primarily on the number of milk samples that are tested at one time. Some kits require that a control sample be used along with the tested milk sample(s). If several tests are performed at the same time, the cost of the control is averaged out across several tests.
5. Each kit will have an expiration date printed on the package or the chemicals within the package. Be sure there is enough time to use the kit before the chemicals expire.
6. Keep kits refrigerated between use, but do not allow the chemicals to freeze. Always allow the kits to warm up to 60 to 70 before testing the milk samples. *Temperature is critical!*
7. Follow directions carefully, and do not take shortcuts. There is no sense in spending the time and money to use this technology if the tests are not performed correctly to ensure accurate results.
8. Most kits use a change in color to determine the level of progesterone. Occasionally the intensity of the color will not be noticeably different from the control. This may indicate the level of progesterone is borderline between high and low.
9. The threshold level *cut off* between low and high progesterone varies among lots. Some kits require that foremilk strippings be used, and others postmilk strippings. The concentration of progesterone may be twice as much in postmilk compared to foremilk. It is essential to use the type of milk specified for the kit used.
10. Although the time required to conduct the test varies between 3 and 22 minutes for the different kits, extra time is required to allow the chemicals to warm up. Allow for this extra time and devote adequate time to conduct the test properly.
11. The time interval from the decline in progesterone to ovulation is variable among cows. This tool should not be used to precisely time artificial inseminations.
12. Low progesterone 19 to 23 days after insemination is an accurate indication the cow is open. However, high progesterone obtained during this time is not an absolute sign the cow is pregnant. Several studies have shown it to be 75 percent accurate in determining if the cow is definitely pregnant. But several conditions other than pregnancy can cause progesterone to be high 19 to 23 days after insemination.

Milk progesterone analysis may be performed on the farm or in a veterinary clinic, but users must become familiar with the procedures, proper interpretation of results, and limitations of this tool. With rapid changes occurring in cow-side diagnostic technology, dairy producers and veterinarians should strive to remain current on the advantages and limitations of such technology.

DISTRIBUTORS OF MILK PROGESTERONE TESTS

Target

Biometallics, Inc.
 P.O. Box 2251
 Princeton, NJ 08543
(609) 275-0133
 12 test kit \$49; 20 test kit \$75

Accufirm

ImmuCell, Inc.
 56 Evergreen Drive
 Portland, ME 04163
(800) 444-4614
 24 and 48 tests per kit

EstruCHEK

Synbiotics Copt.
 11011 Via Front Ers
 San Diego, CA 92127
(800) 247-1725
 36 tests per kit; Microtiter Well
 Useful for clinic; impractical for farm

Characteristics of Commercial Milk Progesterone Kits

(summarized by Ray Nebel, Virginia Tech)

<i>Tradename</i>	<i>Time Required</i>	<i>Ease of Use Index*</i>	<i>Type of Sample</i>
Accufirm-RPT	5 minutes	3	postmilk strippings
EstruCHEK	22 minutes	5	foremilk
Target	10 minutes	2	foremilk

*Scale 1 to 5, with 1 being the easiest

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Issued in furtherance of Cooperative Extension Work, Acts of Congress May 8 and June 30, 1914, in cooperation with the U. S. Department of Agriculture and the Pennsylvania Legislature. T. R. Alter, Director of Cooperative Extension, The Pennsylvania State University.