

The Use of Cowside Milk Progesterone Assay as an Effective Management Tool

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Introduction

Progesterone is a hormone that is produced by the corpus luteum during its functional period in the estrus cycle of the cow. Progesterone is produced by the functional corpus luteum during pregnancy. It can also be produced by a luteal cyst. Progesterone circulates in the blood and can be found in the milk in measurable quantities. The level of progesterone in a milk sample indicates the presence or absence of progesterone producing structures on the ovaries. Determination of ovarian structures at a specific time provides valuable information about the stage of estrus, pregnancy status, and ovarian health, as this article will discuss in detail. A cowside milk progesterone assay can be used to rapidly and economically determine the approximate level of progesterone in a sample of milk, which yields valuable information about the reproductive status of the cow.

Determination of Estrus

It is often difficult to determine, by observation only, which cows are in heat. Some cows undergo silent estrus or only show secondary signs of estrus, while other cows may act as if they are in heat when they are actually in mid-cycle. A normal cycling cow will have a very low level of progesterone in the milk at estrus, and a higher level in mid-cycle. The milk progesterone test can be used to determine if the milk level of progesterone is low or elevated, and thereby determine if the cow is in estrus or in mid-cycle.

Determination of Pregnancy

A normal adult cow has a 21 day estrus cycle. If the cow was bred at estrus, but if conception did not occur, then the cow should be in estrus 21 days later. Milk progesterone level would therefore be very low on day 21. However if conception occurred on day 0, the cow would have a functional corpus luteum at day 21 and milk progesterone would be at a high level. Using the milk progesterone test at day 21 would therefore determine if the cow is pregnant or open. Rectal palpation to determine pregnancy can be performed at about day 30 to day 35. If the cow is found to

be open at day 21 by the milk progesterone test, she can be rebred without having the additional days open. If the cow is identified as pregnant by the milk progesterone test, she should still be checked by rectal palpation to confirm pregnancy.

Identification of a Luteal Cyst

If a cystic structure is palpated on the ovary of a non-cycling cow, the milk progesterone test can be used to determine if it is a follicular or luteal cyst. A luteal cyst produces progesterone, whereas a follicular cyst does not. The milk progesterone test allows a person to correctly identify the cyst and to select the appropriate therapy. A luteal cyst can be treated with prostaglandins, whereas a cyst that is identified as a follicular type can be treated successfully with GnRH.

Determination of Synchronicity in Embryo Transfer

When performing embryo transfer work, it is very important that the donor and recipient cows be synchronized in their estrus cycles. Donor cows are chosen based on the presence of a functional corpus luteum that is responsive to prostaglandins. The milk progesterone test can identify cows with functional corpora lutea that would be candidates for recipient cows.

Milk Progesterone Tests

A composite milk sample is taken from all healthy quarters of the cow's udder. If the sample is not tested within a short period of time, a preservative such as potassium dichromate can be used. Milk samples are evaluated by one of the following tests: The direct radioimmunoassay (RIA) is a laboratory test, and is considered the "Gold Standard" for milk progesterone assays. The field tests use two methods of analysis, the Enzyme Linked Immunosorbant Assay (ELISA) and the Amplified Enzyme Linked Immunosorbant Assay (AELIA). Most field tests are qualitative in nature, and results are determined by observing a color change in the test well compared to a control well. A darker color in the sample well indicates a lower level of progesterone, and a lighter color indicates a higher level of

progesterone. In general, a milk progesterone level greater than 5 ng per ml is considered a high level, and a level of less than 2 ng per ml is considered a low level. Levels of between 2 and 5 ng per ml are considered marginal. Various numbers are used in different brands of kits as a cut-off level between high and low progesterone. These levels range between 2.97 and 4.97 ng per ml.

Products Currently Available

Over the past seven years, many milk progesterone kits have been put on the market, but few are still available. The author knows of three tests that are currently available: the Cite Probe from Idexx Corporation, the Accufirm Rapid Progesterone Test from Immucell Inc., and the Bovine Estrus Slide Test from Angenics, Inc.

Effectiveness of the Milk Progesterone Test

Various studies have been conducted to determine the effectiveness of the milk progesterone test to measure milk progesterone levels and to determine pregnancy status in cows. Basically two types of studies have been done. The first was where results of the cowside milk progesterone tests were compared to results of the radioimmunoassay, and the second where the milk progesterone test was used to predict pregnancy status in cows.

In the first type, the milk progesterone test results correlated highly with the results of the radioimmunoassay when using a high quality cowside kit. Of the milk samples containing low levels of progesterone as determined by RIA, the cowside tests identified greater than 99.4 percent of them as low progesterone samples. Of the milk samples containing elevated levels of progesterone as determined by RIA, greater than 88.9 percent were identified as high progesterone samples by the cowside tests.

Several studies have been done of the second type, to ascertain the efficacy of the milk progesterone assay in pregnancy diagnosis. The ability of the test to determine that a cow was not pregnant is better than its ability to determine that a cow was pregnant. Of the cows predicted to be open, between 92 and 100 percent of these cows were actually open. Of the cows predicted to be pregnant, between 75 and 92 percent of these cows were actually pregnant. An overall accuracy for the milk progesterone test was calculated to be 87 percent, which was an acceptable value when the economic impact of using the milk progesterone test is considered.

A discussion of the errors involved in pregnancy diagnosis with the milk progesterone test, and the economics of using the test will follow.

Problems Associated With the Use of the Milk Progesterone Test

In some cases, the milk progesterone test will fail to provide the correct determination. One such case is when a cow is diagnosed as pregnant by the milk progesterone test at day 21, but early embryonic death occurs. Upon rectal palpation, the cow is determined to be open, and the diagnosis of pregnancy made by the milk progesterone test is found to be incorrect. Early embryonic death is one reason why it is important to check all cows by rectal palpation to confirm pregnancies. Another case where the test will fail is when the cow is bred at the wrong time. If the cow is bred in mid-cycle when a corpus luteum is present, then 21 days later when the cow is tested, the milk progesterone will be high and this will falsely indicate that the animal is pregnant.

There are other situations where the milk progesterone test will not provide the correct determination. If a cow is bred during estrus but does not settle and the cow has a very short estrus cycle, then on day 21 she will be in mid-cycle and milk progesterone will be high, falsely indicating pregnancy. A pathological condition may exist where a progesterone producing structure such as a cyst or a luteal structure is retained through estrus. In this case if a cow is bred on day 0 but does not settle and the cow has a retained progesterone producing structure at day 21, then the cow will be falsely called pregnant at day 21 by the milk progesterone test. The percent butterfat in a milk sample has been postulated to affect the concentration of milk progesterone. In samples with low butterfat, the progesterone concentration may be lower than expected. A final source of error is human inability to detect a color change, or inability to follow the kit directions correctly.

Economics of the Milk Progesterone Test

In one study, milk progesterone assays were used to improve heat detection and therefore decrease the calving to conception interval. A reduction in number of days between calving and conception from 115 to 84 days in one herd and from 85 to 74 days in another yielded a benefit to cost ratio of 7.4 to 1 and 3.4 to 1. A study of the use of cowside milk progesterone assays at breeding time and three weeks later to predict pregnancy showed that for every dollar spent, a return of at least one dollar and eighty three cents could be gained.

A herd manager in Texas was successfully using the milk progesterone test on Mondays to identify cows in mid-cycle. These cows were treated with prostaglandins on Monday, inseminated on Thursday, and checked with the milk progesterone test 21 days later. Cows that were determined to be pregnant were later palpated to confirm their pregnancy status, and cows that were determined to be

open returned to the group that would be checked to identify midcycle cows the following Monday. While not the system of choice for every manager, in this case time spent on heat detection was saved, weekends were freed from heat detection or insemination, and the pregnancy rate doubled.

The cost of the cowside milk progesterone test is about three dollars per kit. This is a minimal investment, considering the potential economic losses such as the cost of semen wasted by breeding at the wrong time. This is especially important when using bulls with very expensive semen. Another important consideration is the time lost between when a person can determine that a cow is not pregnant with the milk progesterone test, and when the cow is palpated and identified as open. This can total between 9 and 25 extra days open, if the milk progesterone test is not used. Each day that a cow remains open represents lost income. These losses can be minimized or eliminated by using the milk progesterone test.

Conclusion

The cowside milk progesterone assay is a simple, fast, inexpensive test that can be used with reasonable accuracy to predict stage of estrus cycle, ovarian pathology, and pregnancy status in cows. The test should be used before breeding and at 21 days post-breeding to maximize its effectiveness and provide the greatest economic return to the user. The test should in no way be thought of as a substitute for excellent management, but as an important adjunct to proper heat detection and pregnancy diagnosis by rectal palpation.

Editor's Note: James Casetta was awarded the first prize (\$200) for his student clinical paper.

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