Milk Progesterone Assays in Pregnancy Diagnosis

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Early pregnancy diagnosis (22 to 26 days postbreeding) by radioimmunoassay of the level of a hormone in milk seems remarkable to most dairymen. Veterinarians are generally less impressed as the same information can be obtained just as early by examination of the reproductive tract per rectum. Dr. Zemjanis[1] in his book, *Diagnostic and Therapeutic Techniques in Animal Reproduction,* has a few words on this subject:

> "Although a definite pregnancy diagnosis is impossible prior to the first expected estrus after service, significant information in regard to conception can be obtained from examination of ovaries and uterus during the time of the first expected estrous period following mating. Thus, finding of a quiescent uterus and fully developed corpus luteum in an animal serviced 20 to 22 days previously is highly suggestive that it has conceived . . . If the uterus exhibits edema simultaneous with tonus and examination of ovaries reveals evidence for recent ovulation, the examined animal is nonpregnant and must be observed closely for signs of heat during the time of expected next estrous period. Thus the outcome of services can be assessed at a relatively early stage. Follow-up examminations conducted in this clinic indicate that the prediction of the outcome of mating based on examination of bred animals 20 to 22 days after service has 85 to 90 percent accuracy. At the present time it might not be practical to schedule visits to herds for examination of all bred animals following mating. This category of animals should, however, be included in the animals to be examined at the regular examination visits."

The results of several studies[2,3,4] on the accuracy of early pregnancy diagnosis by determination of milk progesterone levels are shown in Table I. Thus, both methods are reported to be of similar accuracy, approaching 100 percent for the nonpregnant cow and 85 to 90 percent overall if both pregnant and nonpregnant diagnoses are combined. This is not surprising as pregnancy diagnosis by palpation 20 to 22 days following breeding depends on the presence or absence of a corpus luteum and/or changes (edema and tonus) related to its presence or absence. The presence and amount of progesterone in the milk is also a function of the presence or absence of a corpus luteum and/or its age. This is shown graphically in Figure 1 which depicts the variation in progesterone content of the milk of a normally cycling cow.

The level of milk progesterone decreases as the corpus luteum ages reaching a very low level 1 to 4 days prior to and during estrus. Following ovulation the level remains low for approximately 4 days and then increases as the amount of luteal tissue increases until typical diestrual levels are obtained approximately 12 days after ovulation. Thus, by timing the collection of a milk sample for progesterone analysis or by palpation one can distinguish between the nonpregnant and pregnant cow. As Dr. Zemjanis indicated, it is not generally practical to palpate each cow 20 to 22 days following breeding; however, the ease of collecting a milk sample renders this method of early pregnancy diagnosis practical.

The diagnosis of nonpregnancy approaches 100 percent accuracy; therefore, this test is best termed an early test for nonpregnancy. Its usefulness is the early identification of the cow that is not pregnant but has not been observed in estrus by the time of sampling; generally 24 days after breeding. Eighteen percent of the samples submitted to our laboratory are diagnosed as nonpregnant. This is not surprising as a 30.8 percent incidence of anestrus in dairy cattle has been reported[5] following services not resulting in pregnancy.

Approximately two percent of our samples fall into an arbitrarily defined questionable zone. The dairyman is advised to observe these cows closely for return to estrus. In our experience the majority of these cows are indeed nonpregnant and do return to estrus.

The remaining 80 percent of the samples submitted to our laboratory are diagnosed as pregnant but approximately 15 percent of these return to estrus. As milk progesterone levels are only indicative of the presence of a corpus luteum, errors in the timing of sampling, cow identification, or pathologic conditions resulting in retained corpora lutea undoubtedly account for some of the false positive diagnoses. However, when cow identification and timing of sampling are accurate, early embryonic loss accounts for the majority of the false positive diagnoses. Early embryonic loss has been studied by the sequential collection of milk for progesterone analysis during the early stages of gestation. Several investigators[6,7,8] have reported embryonic losses to be in the range of 7 to 12 percent during the first 60 to 75 days of gestation. Most of these losses occurred prior to day 50 of gestation with means of 46.7 days[8] and approximately 35 days[6] reported in two separate studies. Thus, the period of greatest embryonic loss encompasses the periods of organogenesis, approximately day 14 through day 50 of

gestation, and initial attachment of fetal membranes to the uterine wall. In one trial[6] there was an increasing percentage of early embryonic loss with advancing age of the cow although this was not observed in a subsequent study.[8] From these studies it can be concluded that the cow diagnosed pregnant by the milk progesterone assay should be palpated at approximately 50 days of gestation to affirm the continued presence of a conceptus.

There is also a marked herd-to-herd variation in the percentage of false positive diagnoses. Undoubtedly, trichomoniasis or vibriosis in a herd would increase the percentage of false positive diagnoses as do errors in timing of sampling and cow identification. An interesting possibility is a difference in incidence of embryonic loss between individual bulls not related to a known disease or seminal quality. This possibility was evaluated by the sequential determination of milk progesterone levels during early pregnancy in a herd[8] experiencing a 22 percent indicence of early embryonic loss. Cows bred to one of the bulls used in this herd had a significantly greater (44 percent) incidence of early embryonic loss as compared to the embryonic loss of four comparable herd bulls (approximately 11 percent) used during the same time period. Detailed evaluation of other herds identified by milk progesterone testing as having a high incidence of early embryonic loss may identify similar problem bulls or as yet unrecognized diseases affecting this stage of gestation.

As the amount of progesterone in the milk is indicative of the presence or absence of a corpus luteum, this assay can be used as an adjunct to palpation. Particularly by the beginner, the test can be used to simply verify the presence or absence of a corpus luteum. In a recent study[9] it was shown that the level of plasma progesterone which is highly correlated to the level of milk progesterone and the palpator's findings were in agreement 77 percent of the time. They tended to be in nonagreement during the early or late stage of the cycle. Plasma progesterone was elevated early in the cycle prior to the presence of a palpable corpus luteum and late in the cycle a corpus luteum was palpable after plasma levels of progesterone had fallen to low levels. These discrepancies would not have adversely affected predictions of impending estrus or recent estrus based only on clinical exam. The experienced palpator may find this assay useful in selected cases where the reproductive tract is extremely difficult to retract or multiple cystic structures confuse interpretation.

Another clinical use of this test is evaluation of the accuracy of estrous detection. Several studies[10,11,12] have shown a marked variation in the percentage of the cows in a particular herd that are inseminated when not in estrus. This can be as high as 26 percent of the cows bred.[10] The method of estrous detection (i.e. visual, pressure sensitive device applied to the tail head, testosterone treated marker animal or a combination) was also shown to affect accuracy.[12] With rare exceptions the cow in estrus will have very low levels of progesterone in the milk. Therefore, the accuracy of estrous detection can be readily evaluated by collecting milk from cows on the day of breeding.

At the time of this writing, this service is being offered by

Figure I

Milk Progesterone Levels In a Normally Cycling Cow

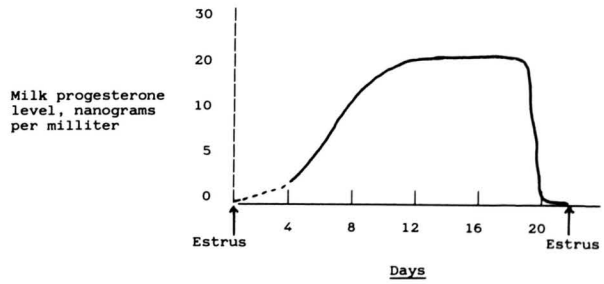

Table I

Accuracy of Pregnancy Diagnosis Determined By Milk Progesterone Levels

Interval Postbreeding (Days)	Pregnancy Diagnosis % Accurate	Nonpregnancy Diagnosis % Accurate
22-26	85.2*	100*
21-22	73.0	98
24	78.0	100

* Does not include herds in which cow identification was a problem.

at least three separate laboratories in the United States--Cornell University College of Veterinary Medicine's Diagnostic Laboratory at Ithaca, New York; Midstates Laboratories at Hillsborough, Wisconsin; and, Texas Veterinary Medical Diagnostic Laboratory at College Station, Texas. These labs charge from $2.50 to $3.50 per sample primarily on a prepaid basis. The dairyman receives all necessary equipment including a prepaid mailer for return of the sample to the lab. Depending on the lab, the dairyman is asked to submit either whole milk or milk strippings. Foremilk is not acceptable as progesterone content is variable but generally low.[13] The milk is preserved during shipment with potassium dichromate.

This test was originally developed in England where it has been offered nationwide for approximately five years. The largest English laboratory is now performing over 100,000 of these determinations yearly and reports the typical dairy utilizing this service is larger than the nationwide average.[14] This laboratory also reports that use of this test increased requests for fertility-related veterinary assistance.[15]

References

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