Field Evaluation of Milk Progesterone Kits for Determination of Pregnancy in Dairy Cows

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One of the new management tools available to dairymen and veterinarians is the milk progesterone test. Tests for concentrations of progesterone in milk have been available for several years but have not been used as extensively in the United States as in other countries.

One type of test utilizes a procedure known as radioimmunoassay (RIA). This test requires laboratory facilities to handle radioactive tracers used for determining amount of progesterone. In addition, sample analysis takes three to seven days. A procedure known as enzyme-linked immunosorbent assay (ELISA) has been developed in more recent years and results in a colored reaction that can be read visually or by an electronic scanner. The ELISA test can be used on the farm, is easy to read and requires less than two hours to develop. ELISA tests for milk progesterone concentration are designed to show whether progesterone levels are "high" or "low" rather than being quantitative as in RIA procedures.

Progesterone is a hormone that is produced by a structure on the ovary known as the corpus luteum (CL). Progesterone has been referred to as the "hormone of pregnancy" and is secreted into the blood, circulated throughout the body, and subsequently secreted into the milk. The CL forms at the ovarian site where the follicle ruptured (ovulation) and released an egg. At the time of ovulation, the cow exhibits signs of estrus (heat). Progesterone produced by the CL increases about four days after estrus, remains high for approximately 16-18 days after estrus in nonpregnant cows and then declines rapidly before the next estrus and ovulation (Fig. 1). The decline in progesterone at the end of the estrous cycle is indicative of CL regression. If the cow has been mated or inseminated at estrus resulting in fertilization of the egg, and pregnancy is successfully established, the CL will be maintained and progesterone production will remain elevated throughout the 280-day gestation period (Fig. 2).

Since progesterone is circulated throughout the body and appears in milk, the ELISA assay can be used to determine milk progesterone concentrations. Because progesterone concentrations at 20-24 days after previous estrus will be lower in non-pregnant cows than in pregnant cows, determination of milk progesterone levels at this time can be used to diagnose pregnancy.

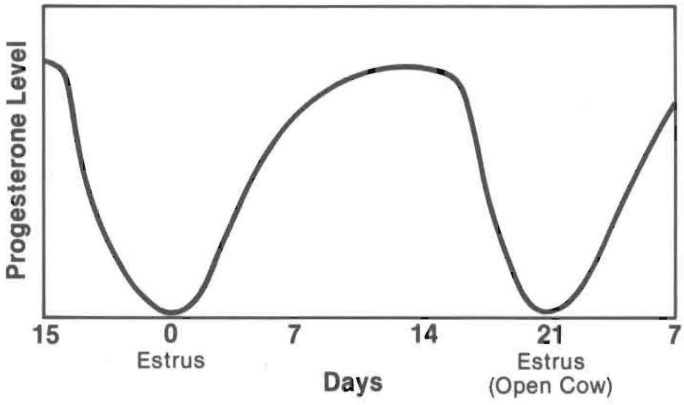

Figure 1. Milk or Plasma Progesterone Levels During Normal Estrous Cycle.

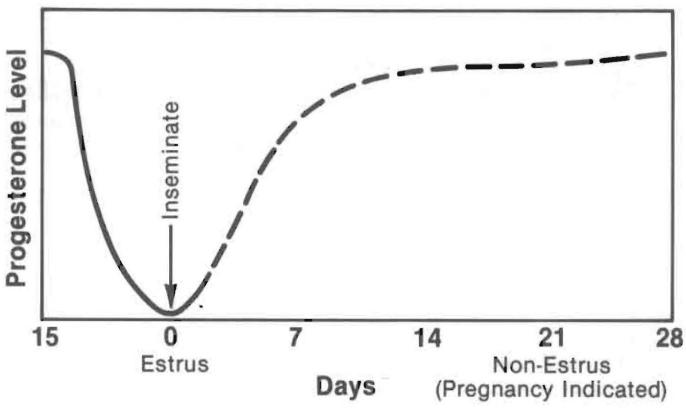

Figure 2. Milk or Plasma Progesterone Levels During Pregnancy.

The objective of this study was to determine if commercially available milk progesterone kits are accurate for determining pregnancy. Milk progesterone levels were determined by using three commercially available kits and compared with standard laboratory RIA analysis of actual progesterone concentrations.

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ELISA procedures for progesterone determination use antibodies that bind and hold progesterone molecules. Progesterone antibodies in some kits are attached to the inside of a plastic tube, fabric dipsticks or wells. When milk is added to the tube, only milk progesterone molecules attach to the antibody and a reagent is added that contains a progesterone-enzyme complex. This complex will attach to antibodies which are not already occupied by milk progesterone. Therefore, the more progesterone in the milk, the fewer antibodies are available for progesterone-enzyme complex binding.

After a short period of incubation with the progesterone-enzyme complex, the tube is emptied and gently rinsed. An enzyme substrate is then added which is modified by the enzyme present in the progesterone-enzyme complex. The more progesterone-enzyme complex in the tube, the more substrate that will be modified. Finally, a developer is added to the tube. The developer reacts only with substrate that has been modified by the progesterone-enzyme complex to produce a colored reaction. Intensity of color is inversely proportional to the amount of progesterone of milk. A nonpregnant cow (low progesterone) will yield a dark color whereas a pregnant cow (high progesterone) will have only a slight color change at 20-24 days after estrus.

METHODS

Three commercially available kits (Accufirm by KaMar Marketing Group, Inc., Box 773838, Steamboat Springs, CO 80477; DiaSystems Ovucare by Tech America Group, Inc., Elwood, KS 66024; and Progestassay by Pitman-Moore, Inc., Washington Crossing, NJ 08560[1]) for determining milk progesterone were evaluated and compared with results obtained by quantitative laboratory RIA procedures for concentrations of progesterone in milk and plasma. Ten cows were bred by artificial insemination at the second or third estrus postpartum. Twenty-one days after insemination, a sample of milk was obtained immediately after morning milking in conjunction with a venous blood sample (tail vein). Milk samples were refrigerated and the three kits were used (within three hours of obtaining milk) to evaluate level of milk progesterone and determine pregnancy status. The remaining milk was frozen. Venous blood was centrifuged, and plasma was decanted and frozen. Milk and plasma samples were analyzed for progesterone by RIA. Rectal palpation was used to confirm pregnancy status of each cow at day 65 after breeding.

Differences in progesterone concentrations between nonpregnant (open) cows and pregnant (settled) cows were analyzed by paired t-test.

[1] *Mention of trade names or proprietary products does not constitute a guarantee or warranty by NDSU or the authors and does not imply approval to the exclusion of other products that may be available.*

RESULTS AND DISCUSSION

Milk progesterone concentrations for nonpregnant cows were less than those of pregnant cows (Tables 1 and 2). Plasma progesterone concentrations also were less in nonpregnant than in pregnant cows (Tables 1 and 2). ELISA kits were very accurate in diagnosing pregnancy status on day 21 after breeding. Quantitative RIA procedures were in full agreement with the ELISA kits (Tables 1, 2).

Several factors could affect the accuracy of the kits and thus determination of pregnancy status. 1) It is necessary to obtain a post-milk stripping sample to minimize the risk of contamination from dirt and manure. 2) The ELISA tests should be performed no later than four hours after collecting the sample. 3) Samples should be stored in a refrigerator or on ice. Progesterone level is affected by other factors such as resorption of the fetus. Embryonic death can be as great as 20 percent to 40 percent during the first 30 to 40 days of pregnancy. Testing an additional milk sample on days 40 to 50 after breeding will confirm maintenance of pregnancy past this critical period.

The ELISA test is also useful in checking the accuracy of estrus detection. If progesterone is high on the day of breeding, it is unlikely that the cow was in estrus on that day. The ELISA test could also be helpful in confirming palpation results on follicular or luteal cysts. A luteal cyst produces progesterone and could be regressed by treatment with prostaglandin $F_2\alpha$. A follicular cyst can be treated with GnRH. In addition, ELISA test for milk progesterone could be used to determine reproductive status in cows exhibiting silent estrus.

Table 1. Progesterone analysis from nonpregnant (open) cows.

Cow	Accufirm	DiaSystems	Progest-assay	RIA ng/ml	
				Milk	Plasma
1163	E	E	E	0.2	ND
1272	E	E	E	0.2	ND
666	E	E	E	ND	ND
5042	E	E	E	0.7	ND
1299	E	E	E	ND	ND

E = Estrus (open) color
ND = Nondetectable

Table 2. Progesterone analysis from pregnant cows

Cow	Accufirm	DiaSystems	Progest-assay	RIA ng/ml	
				Milk	Plasma
5046	P	P	P	5.6	10.8
637	P	P	P	4.0	12.6
1234	P	P	P	4.7	5.6
1236	P	P	P	9.1	7.8
1310	P	P	P	5.8	7.2

P = Pregnant color