The Practitioner's Use of a Rapid Progesterone Assay in the Dairy Cow

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Introduction

Milk progesterone analysis has been available on a commercial basis for dairy producers since the early 1970's. The methods employed at that time were based on Radioimmunoassay (RIA) techniques. Time required for results using this technique was relatively long which resulted in very little acceptance of the procedure for monitoring reproductive status in cattle. Recently, Enzymeimmunoassay (EIA) techniques have been developed for use with milk and plasma. These tests have shown good specificity and a high correlation with RIA techniques (1-6). The advantages of the EIA over current RIA techniques include ease of performing the test, relatively inexpensive equipment required, and rapid results. An EIA for progesterone in milk and plasma (a) has been available in our laboratory since late 1984 and is being used for clinical management of reproduction cases in dairy operations currently participating in our dairy reproductive herd health program.

Principles of the Test

The corpus luteum is a temporary endocrine structure which secretes progesterone during most of the estrous cycle and during pregnancy in the cow. The concentration of progesterone can be monitored by analyzing samples of milk or plasma. The EIA is based on producing antibody specific to progesterone by linking the steroid hormone to a protein. The wells of the microtiter plate are then coated with this antibody. During the assay procedure, the sample (unknown) and standards (knowns) are placed in the wells along with enzyme-labeled progesterone. The labeled and un-labeled progesterone are incubated to allow these substances to compete for antibody binding sites in proportion to their relative concentration. The higher the concentration of the unlabeled progesterone, the less enzyme-labeled progesterone will bind. The assay procedure is concluded by adding a reagent which combines with the enzyme-labeled progesterone to induce a color change. The more intense the color change, the less non-labeled progesterone is present. A spectrophometer is then used with the quantitative test to measure the absorbance value for each well. Progesterone values are determined from a curve created using absorbance values for the standards.

In addition to a quantitative test which utilizes a spectrophometer, there is a quantitative test (cow-side) test (b) available which a dairy farmer can utilize to establish whether progesterone values are "high" (greater than 10 ng/ml milk), "questionable" (5 to 10 ng/ml milk), or "low" (less than 5 ng/ml milk). This test is based on visually assessing the amount of color changes in a series of samples compared to standards. This test requires no specialized equipment and results obtained by the dairyman are highly correlated to the quantitative test results (unpublished data).

Collection of Samples

A small amount of milk is all that is required for performing the test. This can be collected either from the collection bowl of the milking unit or from any disease free quarter. Usually the quarter collection is made prior to application of the milking machine. If the sample is collected from a quarter, 4 to 5 "strips" should be removed from the quarter before the sample is collected.

Current Clinical Investigation

An investigation is currently being conducted at the University of Missouri College of Veterinary Medicine involving 4 dairy herds whose management includes a routine veterinary reproductive herd health program. The qualitative "cow-side" test is run by each of the dairymen at his dairy, and its results noted. The samples are kept at the dairy until the next dairy herd visit. These samples are returned to the laboratory where they are assayed using the quantitative plate reader method. Several thousand samples

a) OVUCARE™ Supplied by Cambridge Life Sciences, Cambridge Science Park, Milton Road, Cambridge, England CB4 4BH.

b) OVUCHECK™ Supplied by Cambridge Life sciences, Cambridge Science Park, Milton Road, Cambridge, England CB4 4BH.

have been assayed in this manner and the research is still in progress.

Clinical Applications

The clinical uses of progesterone analysis include:

1. Identification of non-pregnancy
2. Evaluation of anestrous cows
3. Confirmation of estrus
4. Response to therapy

Identification of Non-Pregnancy

The principle applied to identification of non-pregnancy in the cow is based on levels of progesterone present during the normal estrous cycle. Progesterone levels are low during estrus which occurs at approximately 21 day intervals in the cow. There has been general agreement in the literature that milk samples collected and analyzed at 20-24 days after service are 70 to 80% accurate in detecting pregnancy and nearly 100% accurate in identifying non-pregnant cows (7-12). Progesterone analysis is therefore much more accurate for detecting non-pregnancy than pregnancy. It is important that the veterinarian emphasizes that analysis of milk samples collected at the first expected postservice estrus is a means of accurately identifying non-pregnant cows instead of as an indicator of pregnancy. Cows which have high milk progesterone concentrations at 20 to 24 days after insemination may be pregnant (70 to 80%) or the test may be a "false positive" due to a number of causes, some of which are:

a. Insemination during the luteal phase
b. Long or short estrous cycles
c. Prolonged luteal lifespan (uterine pathology)
d. Embryonic death (after day 16 or 17)
e. Ovarian cysts
f. Management errors (mistiming of sample, mistaken identification of cows and/or samples
g. Laboratory errors

In an effort to gain information on how many milk samples are needed to identify the non-pregnant animal, samples were collected on the day of breeding and days 17, 18, 19, 20, and 22 post-breeding. If progesterone levels were elevated on the day of breeding, the breeding was disregarded and not considered in the evaluation of the data. If progesterone levels remained high during the post-breeding interval, the animal was listed as *possibly pregnant* and scheduled for examination on approximately day 40 of gestation. If the progesterone level was below 5 ng/ml, the animal was listed as non-pregnant. The statistical analysis of this data has not been completed, but preliminary indications are as follows:

a. Approximately 6% of the animals bred are not in true estrus.
b. 99% of the cows found with low progesterone on any day are nonpregnant. (Close to 100%)
c. 27% of the cows listed as possibly pregnant on day 22 are open.
d. Recognition by the dairyman of a lowered progesterone level in a cow has intensified the detection of behavioral signs of estrus. All producers involved in this study were in agreement.
e. When several animals are exhibiting signs of estrus, it aids in detection of true estrus.

Evaluation of Anestrus Cows

Background

During routine reproductive herd health visits, the most common classification of cow examined is for anestrus (cows which fail to show estrus). It has been reported that 90% of the cases of anestrus are due to failure in observation of estrus and 10% are actually due to pathological conditions (13). This failure to detect estrus is the major cause of infertility in dairy herds with twice as much time lost due to failure to detect estrus as is due to conception failure (14). In an attempt to reduce the problem of unobserved estrus (pre-breeding anestrus), Ball and Jackson (15) measured milk progesterone using RIA techniques in a herd of cows which were observed for signs of estrus six times per day. Cows which exhibited estrus were used as controls and were inseminated in the usual manner. Cows which did not exhibit estrus were inseminated on the second and third days after a drop in progesterone concentration was detected. They reported that 65.2% of the controls became pregnant and 60.0% of the cows which were inseminated based on progesterone values alone became pregnant. They concluded that cows are capable of conceiving at ovulation even if that ovulation is not accompanied by detection of estrus. They further stated that the use of RIA for breeding cows was not practical for use in routine dairy infertility work. Another study using EIA for the measurement of progesterone to allow insemination of cows not exhibiting estrus was done by Foulkes et al (16). They divided cows into two groups of 82 and had one group (controls) inseminated based on observation of estrus. The treatment group had progesterone values measured daily and were inseminated a single time two days after detecting the characteristic fall in milk progesterone which occurs after luteolysis. The pregnancy rates during the 30 day duration of this experiment were similar in both groups (67.2% in controls and 63.8% in treatment group). However, significantly more cows were inseminated in the treatment group (80 of 82) than in the control group (58 of 82). Also, the mean calving to first service interval was significantly reduced on each farm.

Post-Breeding Anestrus

If progesterone concentrations are measured in milk samples taken on days 17 to 22 after breeding most cows which have failed to conceive can be identified and inseminated at the first expected post-service ovulation whether or

not that ovulation is accompanied by behavioral signs of estrus. Cows not detected in estrus can be artificially inseminated on the day following two consecutive days of low progesterone (the third consecutive day of low progesterone concentration). Studies are currently under way to determine the minimum number of progesterone assays necessary for identification of the maximum number of non-pregnant cows (pre-breeding and post-breeding).

Pre-Breeding Anestrus

Studies are currently under way to determine effective methods of using progesterone assays in the pre-breeding period. Though not as economically important as post-breeding anestrus, it is commonly thought of as the most important form of anestrus by the producer. It is very important to urge the producer to keep accurate records of all heats during the pre-breeding period. These can serve as a benchmark to begin milk sample collection for monitoring the estrous cycle. A milk sample should be taken on day 19 or 20 post-heat even though the owner may not want to breed the animal.

During the post-partum or pre-breeding anestrus examinations of animals during a herd health visit in which a corpus luteum is palpable on one of the ovaries, the veterinarian can use prostaglandin to produce luteolysis and synchronize the cycle. The cow can either be observed for estrus and bred if desired, or, if prostaglandin therapy is not initiated, the cow can be monitored by weekly milk progesterone assay.

Confirmation of Estrus

Studies have indicated that errors in estrus detection resulted in failures to detect heat and missed opportunities for insemination. These studies also showed that misinterpretation of behavioral signs of estrus result in insemination of 10 to 26% of cows during the luteal phase of the estrous cycle when conception is unlikely (10, 17-21) or the insemination of pregnant cows (22). In herds with a history of poor fertility in which poor heat detection is suspected, assay of progesterone in milk samples taken on the day of estrus can be used to confirm that cows which are to be inseminated are not in the luteal phase of the cycle. If a high percentage of such samples have high progesterone levels, a review of the herds estrus detection procedures is indicated.

Response to Therapy

It is possible to evaluate the reponse of cows to therapy through the use of progesterone assays. In the case of follicular ovarian cysts, luteinization of the cyst in response to GnRH is accompanied by an increase in progesterone production. Cows which have not responded can be identified with progesterone assay and retreated without further loss of time. Prostaglandin F2 alpha may also be used to shorten the interval from GnRH treatment to breeding (23).

In the case of a normal palpable corpus luteum (CL) in a cow which is over 45 to 60 days post-partum, it is often desirable to use prostaglandin F2 alpha to cause luteolysis of the CL so that the earliest possible breeding can take place either on observed estrus or on timed insemination. Plunkett et al (24) found in one study that prostaglandin F2 alpha was effective in causing luteolysis of a palpably mature corpus luteum 91.0% of the time. It is possible to establish the effectiveness of prostaglandin F2 alpha therapy on a practical basis using EIA to establish whether a characteristic drop in progesterone concentration does in fact occur by analyzing a sample taken 3 days after the prostaglandin injection. If progesterone concentrations fail to decline indicating failure to respond to treatment, cows can be retreated and prevent a further delay in breeding.

Summary

The introduction of enzymeimmunoassay for measurement of progesterone concentrations in milk and plasma offer the practicing veterinarian another diagnostic tool which can be combined with his clinical skills to insure the best response to therapy and reproductive performance currently possible in livestock. It seems difficult to convince the veterinarian of the importance of this technique in routine herd reproductive programs. The producer has expressed an intense interest in this technique, however. It will be very important for the veterinarian to become familiar with this technique so that he can aid the dairyman in the interpretation of results. The veterinarian must emphasize that progesterone concentrations in milk and plasma reflect only the presence or absence of functional luteal tissue and an accurate biological test for pregnancy in the cow must await the development of practical assays for a substance specifically associated with the embryo or pregnant uterus such as early pregnancy factor (25). Presently, the best and most accurate means of pregnancy detection in the cow is rectal palpation by an experienced veterinarian.

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