Timing is everything in pregnancy diagnosis

Blood and milk samples are both options for pregnancy diagnosis. Pick the option that fits best into your farm's workflow.

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IDENTIFICATION of nonpregnant dairy cows early after insemination, and coupling a nonpregnancy diagnosis with a strategy to rapidly rebreed nonpregnant cows, can improve the pregnancy rate in a dairy herd. This can be accomplished twofold by reducing the interval between A.I. services, thereby raising A.I. service rate.

Methods for early pregnancy diagnosis in cattle can be classified as either direct or indirect. Direct methods involve detection of the tissues and/or associated fluids of the conceptus either manually (through rectal palpation) or via electronic means such as ultrasound.

Direct methods for pregnancy diagnosis have been around longer and are the most popular methods for determining pregnancy status. By contrast, indirect methods for early pregnancy diagnosis use measures of hormones or pregnancy-specific substances in maternal body fluids as indicators of pregnancy. Indirect pregnancy tests recently have been developed based on progesterone in milk; however, the latest indirect pregnancy tests are based on detection of pregnancy-associated glycoprotein (PAG) levels in blood or milk.

What we learned

We recently conducted an experiment to assess factors associated with PAG levels in the blood and milk of dairy cows during early pregnancy. Our goal was to determine the best way to incorporate PAG testing into a reproductive program on a dairy farm.

We synchronized 141 lactating cows to receive their first timed A.I. (TAI). Blood and milk samples were collected 25 and 32 days after timed A.I., and pregnancy status was determined 32 days after timed A.I. using ultrasound. There were 48 cows diagnosed pregnant with a single calf 32 days after timed A.I. that continued the experiment.

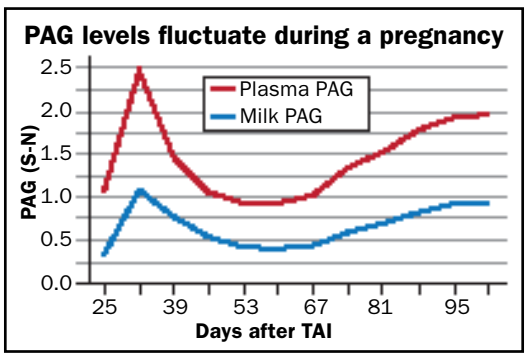
From this group, we assessed blood and milk samples to reconfirm pregnancy status on a weekly basis using ultrasound from 39 to 102 days after TAI. Likewise, plasma and milk samples were assayed for PAG levels using commercial ELISA kits.

The incidence of pregnancy loss for cows diagnosed with singleton pregnancies 32 days after timed A.I. during the experiment was 13 percent (7/55). Again, that left us with 48 pregnant cows in the study. This figure agrees with the 13 percent pregnancy loss reported to occur from 27 to 31 and 38 to 50 days of gestation based on ultrasound in a summary of 14 previous studies.

For the plasma PAG test, all but one cow that underwent pregnancy loss tested positive. Meanwhile, all cows undergoing pregnancy loss tested positive at one or more time points for the milk PAG test. Similarly, five of seven cows tested recheck based on the plasma PAG test before the loss occurred compared to three of seven cows based on the milk PAG test. That means PAG levels detected by these tests have a half-life in maternal circulation resulting in a 7 to 14 day delay in identification of cows undergoing pregnancy loss based on plasma or milk PAG levels compared to ultrasound.

Also, PAG levels are high during late gestation; it takes up to 60 days for residual PAG to be cleared from maternal circulation after calving. Because of the PAG half-life in circulation, cows submitted for a pregnancy test before 60 days after calving can test positive due to residual PAG levels from the previous pregnancy. The manufacturer of the plasma and milk PAG tests evaluated in this experiment recommends that cows be greater than 60 days after calving when tested.

Plasma and milk PAG levels were affected by both week-after timed A.I. (see figure) and parity. When all cows that maintained pregnancy from 25 to 102 days after TAI were analyzed, plasma and milk PAG levels rose from 25 days after TAI to an early peak 32 days after TAI. Plasma and milk PAG levels then fell 32 days after TAI to a low point from 53 to 60 days after TAI for the plasma PAG ELISA and from 46 to 67 days after TAI for the milk PAG ELISA. That was followed by a gradual rise in PAG levels from 74 to 102 days after TAI. First-lactation cows had greater plasma and milk PAG levels than older cows.



The overall accuracy of the plasma and milk PAG tests 32 days after timed A.I. was 92 percent and 89 percent, respectively. Results support the accuracy of using plasma or milk PAG levels as an indicator of pregnancy status in dairy cows 32 days after A.I. Our results agree with others who have conducted similar analyses from 27 to 39 days in gestation when PAG levels in both plasma and milk are at early peak levels.

Which PAG test is best?

Based on the sensitivity analysis in this experiment, both the plasma and milk PAG ELISA tests are accurate for pregnancy diagnosis when conducted 32 days after A.I. based on the plasma and milk PAG profiles (see figure). The choice of whether to use the blood or the milk test is determined by the availability of the test and the ability to collect the samples.

From a practical perspective, neither the plasma nor the milk PAG tests are cowside or on-farm tests. Cows must be identified and restrained to collect a blood or a milk sample. Then, the samples must be sent to an off-farm laboratory that can run the ELISA test. Within several days and after receiving the pregnancy outcome, cows diagnosed not pregnant must again be identified and restrained to submit them to a strategy for rapidly returning them to A.I. This is best achieved as part of an aggressive resynchronization strategy for nonpregnant cows.

Depending on the farm, milk samples may be easier to collect than blood samples. The only commercially available milk PAG test (IDEXX Laboratories, Westbrook, Maine) is marketed through regional DHIA testing centers throughout the U.S., making the test widely accessible to most farms. A pregnancy diagnosis can be easily conducted on the same milk samples sent for DHIA testing on a monthly basis; however, monthly pregnancy examinations are not frequent enough to drive the reproductive program on a dairy farm. This makes it necessary to conduct additional tests on a weekly or bi-weekly basis.

By contrast, many farms can easily collect blood samples, and three commercial blood pregnancy tests are available in North America (BioPRYN, BioTracking LLC, Moscow, Idaho; DG29, Conception Animal Reproduction Technologies, Beaumont, Quebec; IDEXX Bovine Pregnancy Test, IDEXX Laboratories, Westbrook, Maine). The blood ELISA tests are run in regional laboratories located around North America and should be accessible to most farms. Care should be taken, however, to make sure samples are labeled correctly.

Based on PAG profiles in plasma and milk samples collected weekly, the optimal time to conduct a first pregnancy diagnosis is around 32 days after TAI when plasma and milk PAG levels are at an early peak. Remember, conducting either the plasma or milk PAG test during the low point in plasma and milk PAG levels would result in poor overall accuracy. Because of the occurrence of pregnancy loss, all pregnant cows should be submitted for a pregnancy recheck 74 days or later after A.I. when relative PAG levels in plasma and milk of pregnant cows have rebounded from their low point.

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