Pregnancy Diagnosis using Milk PAG Testing

Paul M. Fricke, Ph.D., Professor of Dairy Science and Extension Specialist
University of Wisconsin – Madison

Identification of nonpregnant dairy cows early after AI improves reproductive efficiency and pregnancy rate by decreasing the interval between AI services thereby increasing the AI service rate. Thus, new technologies to identify nonpregnant dairy cows and heifers early after AI may play a key role in management strategies to improve reproductive efficiency and profitability on dairy farms. Chemical tests for early pregnancy diagnosis that use qualitative measures of pregnancy-associated glycoproteins (PAGs) originating from the placenta have been developed and commercialized. Because PAGs are produced specifically by the placenta, the presence of PAGs in blood can be used to accurately determine pregnancy status. Currently, three non-pregnancy tests based on detection of PAGs in maternal blood are commercially marketed:

BioPRYN - BioTracking, LLC, Moscow, ID
http://www.biotracking.com/dairy
DG29 - Conception Animal Reproduction Technologies, Beaumont, QC
http://www.conception-animal.com/test_an.html
IDEXX Bovine Pregnancy Test - IDEXX Laboratories, Inc., Westbrook, ME,
http://www.idexx.com/view/xhtml/en_us/livestock-poultry/ruminant/lpd-bovine-pregnancy-test.jsf

None of the tests listed above are cow-side or on-farm, so blood samples must be collected by farm personnel and sent by courier to a local or regional laboratory that runs the assay. Results are then returned to the farm via email, usually within 24 to 72 h.

Recently, IDEXX Laboratories (Westbrook, ME) released a milk PAG test marketed through regional DHIA testing centers throughout the United States. Detection of PAGs in milk samples eliminates the need for drawing blood samples and can be done at the DHIA testing center on the same milk samples sent in for determining milk components and somatic cell score. Because PAGs have a long half-life in circulation after calving, cows must be a minimum of 60 days post-calving for accurate results. In addition, PAGs increase slowly in milk early in gestation, so cows must be ≥28 days post-insemination for the milk PAG test to be accurate.

We recently conducted an experiment to characterize milk PAG levels throughout the first trimester of gestation in dairy cows and to assess the accuracy of pregnancy outcomes compared to transrectal ultrasonography (Ricci et al., 2014). A total of 141 lactating Holstein cows were hormonally synchronized to receive their first postpartum timed artificial insemination (TAI). Milk samples were collected 25 and 32 days after TAI, and pregnancy status was determined 32 days after TAI using transrectal ultrasonography. Cows diagnosed pregnant with singletons 32 days after TAI continued the experiment in which milk samples were collected and pregnancy status was assessed weekly from 39 to 102 days after TAI using transrectal ultrasonography. The incidence of pregnancy loss from 32 to 102 days after TAI for cows diagnosed with singleton pregnancies was 13%, and these cows were removed from the data set. Thus, a total of 48 cows maintained a singleton pregnancy from 32 to 102 days after TAI. Mean relative levels (S-N

values) of PAGs in milk from pregnant cows are shown in the upper panel of Figure 1. Milk PAGs increased from 25 days after TAI to an early peak 32 days after TAI. Milk PAGs then decreased from 32 days after TAI to a nadir from 46 to 67 days after TAI followed by a gradual increase in PAG levels from 74 to 102 days after TAI.

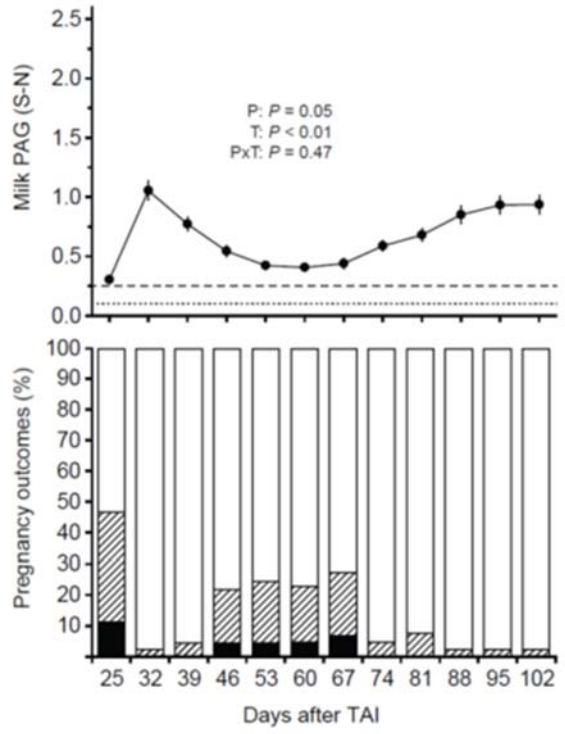

Figure 1. Upper Panel: Relative pregnancy-associated glycoprotein (PAG) levels (S-N values) in milk of Holstein cows from 25 to 102 d in gestation. Lower Panel: Stacked bar graphs of pregnancy outcomes based on PAGs in milk of Holstein cows from 25 to 102 d in gestation. Based on cutoff values determined by the manufacturer, cows were classified as "not pregnant" (black bars) "recheck" (hatched bars); or "pregnant" (open bars).

To determine the accuracy of milk PAG test outcomes during the first trimester of gestation (Figure 1, lower panel), pregnancy outcomes based on PAGs in milk were classified based on cutoff levels (dashed lines in the upper panel of Figure 1) specified by the manufacturer. Overall, pregnancy outcomes for pregnant cows reflected the PAG levels in milk. Clearly, testing at 25 days after AI is too early based on the proportion of "not pregnant" and "recheck" outcomes generated for cows that we knew were pregnant. By contrast, milk PAG test outcomes for pregnant cows exceeded 98% "pregnant" outcomes 32 days and 39 days after TAI for cows we knew were pregnant. The proportion of milk PAG test outcomes of "not pregnant" and "recheck" then increased for cows that maintained pregnancy concomitant to the temporal decrease in milk PAGs during the nadir and then decreased as milk PAGs increased as gestation ensued.

Based on PAG profiles in milk samples collected weekly, the best time to conduct a first pregnancy diagnosis is around 32 to 39 days after TAI when milk PAGs are at an early peak in pregnant cows. Because we only collected milk samples weekly, we are not able to determine the earliest day when milk PAG testing is accurate, so we recommend following the manufacturers recommendation of ≥28 days after AI. By contrast, conducting the milk PAG test during the temporal nadir in milk PAGs from 46 to 67 days after AI would result in a lesser overall accuracy of the test outcomes and the possibility of aborting a few pregnancies if prostaglandin $F_{2\alpha}$ is administered based on "not pregnant outcomes." Finally, because of the occurrence of pregnancy loss, all pregnant cows should be submitted for a pregnancy recheck at 74 days after AI or later when relative PAG profiles in milk of pregnant cows have rebounded from their nadir.

Reference

Ricci, A., P. D. Carvalho, M. C. Amundson, L. Vincenti, and P. M. Fricke. 2014. Accuracy of pregnancy outcomes based on pregnancy-associated glycoproteins in milk and serum during the first trimester of gestation in lactating dairy cows. J. Dairy Sci. 97(Suppl. 1):694.