New Technologies to Improve Dairy Cattle Reproduction

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Synergies between new reproductive management technologies hold the key to maximizing reproductive efficiency on dairy farms; however, reproductive management protocols that allow for synchronization of ovulation and subsequent identification and resynchronization of nonpregnant cows must be practical to implement within the day to day operation of a dairy farm or the protocol will fail due to lack of compliance (Fricke et al., 2003). This is especially true for larger farms that must schedule and administer artificial inseminations, hormone injections, and pregnancy tests for a large number of animals on a daily or weekly basis.

New technologies to improve reproduction discussed in this panel include: 1) use of pregnancy-associated glycoprotein (PAG) tests to determine pregnancy status; 2) use of progesterone testing to assess and manage reproduction; and 3) use of accelerometer systems to detect estrus. This panel will feature three individuals who have extensive practical experience using each of the technologies discussed. Contact information and a biography of each panelist appear before a brief synopsis of each technology and a summary of recent research in each area presented in each section.

Panel Member: **Dr. Robert Vlietstra**, DVM. Contact: robvliet@gmail.com.

Biography: Robert Vlietstra taught Geology, Biology and Math in Midland, Michigan after graduation from Calvin College in 1974. He completed an additional B.S. degree in Animal Science (1978) at MSU and the D.V.M. degree (1982) from the College of Veterinary Medicine at Michigan State University. Bob worked as a resident farm veterinarian in Lexington, Kentucky after completing the DVM curriculum. He was an associate veterinarian in LaGrange, Indiana, as he worked his way back to Michigan. In August of 1983, he began an eleven year stint as a solo practitioner in the Zeeland, Michigan area. Dr. Bob merged his solo practice with a neighboring established practice that began West Michigan Veterinary Service. His professional interest is dairy production medicine with a concentration in theriogenology and nutrition. He serves WMVS as the director of its Reproduction Consulting Service and Laboratory, featuring the Bio-PRYN test.

Use of Pregnancy-Associated Glycoprotein (PAG) tests to assess pregnancy status

Early identification of nonpregnant dairy cows post breeding can improve reproductive efficiency and pregnancy rate by decreasing the interval between AI services and increasing AI service rate. Thus, new technologies to identify nonpregnant dairy cows early after artificial insemination (AI) may play a key role in systematic management strategies to improve reproductive efficiency and profitability on commercial dairy farms. Transrectal palpation is the oldest and most widely used method for early nonpregnancy diagnosis in dairy cattle (Cowie, 1948); however, a newer technology may emerge to replace transrectal palpation as the method of choice for nonpregnancy diagnosis in the dairy industry. One such technology is the development of commercially available tests to detect pregnancy-associated glycoproteins (PAGs) in maternal serum; however, this new technology must be practically integrated into a systematic on-farm reproductive management strategy for it to succeed. Research reviewed in this paper support use of PAGs as a method for early nonpregnancy diagnosis in dairy cattle; however, there are some caveats and limitations with regard to their incorporation into a systematic reproductive management program that must be considered to determine the appropriate timing of nonpregnancy diagnosis.

Pregnancy-associated glycoproteins constitute a family of inactive aspartic proteinases (Xie et al., 1991) comprising 22 genes located on chromosome 29 in the bovine (Telugu et al., 2009) with different patterns of expression throughout pregnancy and produced mainly by the binucleate cells of the placenta (Xie et al., 1991; Green et al., 2000; Patel et al., 2004) but also by the trophectoderm (Xie et al., 1991). Placentation in ruminants is noninvasive and is classified as synepitheliochorial cotyledonary, which describes the fetal-maternal syncytium formed by the fusion of trophoblast binucleate cells and uterine epithelial cells (Wooding, 1992). The giant binucleate cells are large cells containing two nuclei and are the invasive component of the trophoblast representing 15 to 20 % of the total cellular population within the mature placenta. Mature chorionic binucleate cells at all stages of bovine pregnancy migrate into the uterine epithelium and release the contents of cytosolic granules containing PAG's through exocytosis where they enter the maternal circulation (Wooding and Whates, 1980; Wooding, 1983; Zoli et al., 1992).

Identification of nonpregnant cows early post breeding can only improve reproductive efficiency when coupled with a management strategy to rapidly submit nonpregnant cows for a subsequent AI service. Thus, any method for early nonpregnancy diagnosis must be integrated as a component of the overall reproductive management strategy in place on the farm.

Currently, three non-pregnancy tests based on detection of PAGs in maternal serum are commercially available for use by dairy farmers:

1) BioPRYN
BioTracking, LLC, Moscow, ID
http://www.biotracking.com/
2) DG29
Conception Animal Reproduction Technologies, Beaumont, QC
http://www.conception-animal.com/test_an.html
3) 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 these tests 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 has developed a PAG test for milk samples that is marketed through regional DHIA testing centers throughout the United States. Cows must be ≥35 days post-insemination and must be a minimum of 60 days post-calving for accurate results. This milk-based PAG test is currently being marketed as a pregnancy recheck for cows initially diagnosed pregnant by a veterinarian.

Data from a field trial conducted in Wisconsin (Silva et al., 2007b) supported that use of a commercial PAG assay for detecting non-pregnant cows 27 d after AI yielded acceptable sensitivity and specificity, had a high negative predictive value indicating that few cows would be subjected to iatrogenic pregnancy loss during a resynchronization protocol, and had a similar accuracy when compared to transrectal ultrasonography. Furthermore, commercial PAG tests may more reliably detect cows undergoing pregnancy loss compared to use of transrectal ultrasonography when the non-pregnancy test is conducted before 30 d after AI (Giordano et al., 2012). Data from a second field trial in Wisconsin (Silva et al., 2009) showed that initiation of resynchronization 25 d after an initial TAI resulted in similar fertility to initiation of resynchronization 32 d after TAI in lactating Holstein cows thereby decreasing DIM at TAI and total days open after the initial TAI. Based on our results (Giordano et al., 2012), it is important to follow the manufacturer's instructions when using these commercial tests so that cows are not subjected to non-pregnancy testing too early postpartum to avoid false positive results and that cows are not tested too early post-insemination to avoid poor sensitivity, specificity, and accuracy of the test.

Panel Member: **Dr. Neil Michael**, DVM, MBA. Contact: nmichael@vitaplus.com.

Biography: Dr. Michael is Director of Dairy Initiatives at Vita Plus in Madison, WI. In this newly created position, Michael works with the entire dairy team to implement targeted initiatives in dairy nutrition and management. His activities range from consulting on individual dairies to development and implementation of support tools for Vita Plus staff. He serves as a resource for dairy customers and staff on dairy records, nutrition, management and reproductive strategies. Michael graduated with a Bachelor of Science degree from Purdue University. He continued his education at Purdue, earning veterinary certification in 1982 and a Masters of Business Administration in 2006. He practiced veterinary medicine, primarily in Wisconsin, from 1982 to 1997 before working in sales and technical services with Monsanto Dairy Business. For the past 10 years, Michael worked in technical services at ABS Global, Inc. in Deforest, Wis., most recently as Director of Global Technical Services. He is a member of the American Veterinary Medical Association, American Association of Bovine Practitioners, American Dairy Science Association and Wisconsin Veterinary Medical Association.

Progesterone Testing to Manage and Assess Dairy Cattle Reproduction

Progesterone is a female steroid hormone produced by dairy cattle post puberty during specific stages of each estrous cycle and is required for maintenance of pregnancy. Progesterone is produced by the corpus luteum that develops on an ovary after ovulation and is secreted into the blood and subsequently into milk. At the time of estrus, progesterone levels are low in both blood and milk after which levels increase until Day 17 to 18 of the estrous cycle when they decrease again (unless the animal is pregnant) to low levels as the next follicle and estrus approach. As a result of their relationship with estrus and cyclicity, measurement of progesterone in blood or milk can be used as a tool for reproductive management on commercial dairies.

Progesterone can be measured quantitatively in laboratories using an expensive radioimmunoassay (RIA) procedure. A RIA is performed on whole blood or serum samples and is considered the gold standard for P4 measurement. Additionally, ELISA technology can be used to measure progesterone levels quantitatively or qualitatively in milk and blood and is available in both laboratories and cowside applications.

Strategic progesterone testing can help you evaluate heat detection accuracy (Rivera et al., 2004), cyclicity status (Silva et al., 2007a), and synchronization efficiency. Additional applications for blood progesterone evaluation include training and certification of AI technicians as well as setup of new activity system installations.

Blood samples can be obtained from the caudal tail vein in restrained cattle. Either whole blood or serum is acceptable, and samples should be refrigerated before submission to a laboratory for testing.

- *Accuracy*: for chalk or visual detection methods, collect a minimum of 15 samples from animals detected in estrus and inseminated within the last 24 hrs. Samples can be refrigerated until the necessary number of samples is collected for submission to the laboratory. Note that samples should be unannounced to prevent biased sampling.
- *Synchronization Efficiency (SE)*: collect a minimum of 15 samples from animals enrolled within a TAI program on the day of insemination. Additional sample sets may be required to investigate particular bias' such as DIM, parity, or times bred.
- *Cyclicity*: collect a second sample on the SAME animals sampled for Synchronization Efficiency above at 7 to 14 days after insemination. The SE samples should be held until cyclicity samples are taken and the duplicate sets submitted to the lab at the same time.

The accepted cutoff for blood progesterone (P4) levels evaluated by RIA methods is 1 ng/ml. Levels below the cutoff are considered to be indicative of animals near estrus or animals without ovarian activity (non-cyclic) while levels above 1ng/ml are indicative of animals with a source of progesterone production (ie. a corpus luteum). Note that high levels of blood progesterone by themselves should not be considered confirmation of pregnancy status. Using the above methods, interpretation and goals are as follows:

- Accuracy of detection is calculated as the percent of samples collected from cows detected to be in estrus that are low P4 with a goal of greater than 85%.
- Synchronization efficiency is reported as the percent of cycling animals with low P4 divided by the number of cycling animals evaluated with a goal of greater than 95% at time of TAI.
- Cyclicity is calculated as the percent of total sample pairs where P4 levels go from low to high (or high to low) during the 7-14 day sampling interval. Goal for cyclicity is greater than 95% of animals that are submitted to a TAI program although this will differ dependent upon DIM at evaluation.

Panel Member: **Peter Dueppengiesser**, Perry, NY. Contact: dueppdairy@frontiernet.net.

Biography: A 1986 graduate of Cornell University, Peter earned his B.S. in Animal Science. He is a partner in Dueppengiesser Dairy Company in Perry, New York with his brother Mike. They milk 1200 cows and raise 1000 head of young stock. R.H.A. 27,370 3.8 1036 3.0 816. They crop 800 acres of corn, 100 acres of wheat, and 900 acres of alfalfa. The dairy has been using the Semex AI

24 Heat Detection System since June 2010 along with various pre-synch, ov-synch strategies. They are very active in showing and marketing registered Holsteins under the Ransom Rail Farm prefix. Peter served as a past chairman of the Northeast Dairy Producers Association (NEDPA). He volunteers as a leader for his local 4-H program, as an advisor for the junior Holstein club, and serves on the parish council in his local church. Married to his wife Roxanne for 21 years, the couple has two sons, Jacob, a freshman at Cornell University, and Jared, a junior in high school.

Use of Accelerometer Systems for Detection of Estrus

Despite the widespread adoption of hormonal synchronization protocols that allow for timed artificial insemination (TAI), detection of behavioral estrus continues to play an important role in the overall reproductive management program on most dairies in the U.S. (Caraviello et al., 2006; Miller et al., 2007). Several challenges for estrus detection on farms include attenuation of the duration of estrous behavior associated with increased milk production near the time of estrus resulting in shorter periods of time in which to visually detect estrous behavior (Lopez et al., 2004), low number of cows expressing standing estrus (Lyimo et al., 2000; Roelofs et al., 2005; Palmer et al., 2010), silent ovulations (Thatcher and Wilcox, 1973; Palmer et al., 2010; Ranasinghe et al., 2010), and reduced expression of estrous behavior due to confinement (Palmer et al., 2010). Whatever the cause, the low efficiency of estrus detection not only increases time from calving to first AI but increases the average interval between AI services (Stevenson and Call, 1983), thereby limiting the rate at which cows become pregnant.

Because of the impact of AI service rate on reproductive performance and the problems associated with visual estrus detection on farms, many technologies have been developed to enhance estrus detection by providing continuous surveillance of behavior including rump-mounted devices and androgenized females (Gwazdauskas et al., 1990), pedometry (Peralta et al., 2005; Roelofs et al., 2005), and radiotelemetry (Walker et al., 1996; Dransfield et al., 1998; Xu et al., 1998). New electronic systems that incorporate accelerometers as a means to associate increased physical activity with estrous behavior in cattle (Holman et al., 2011; Jónsson et al., 2011) have been developed and marketed to the dairy industry. Whereas a large body of literature exists on the accuracy and efficacy of using various technologies to predict ovulation and timing of AI in relation to ovulation in lactating dairy cows, no other studies have investigated accelerometers for such purposes.

Recent data from the University of Wisconsin-Madison that evaluated an accelerometer system (Valenza et al., 2012) showed that only two thirds of the cows that were considered properly synchronized to come into estrus would have been inseminated based on the accelerometer system and would go on to ovulate after AI. The remaining cows either would not be inseminated because they were not detected in estrus or would not have a chance to conceive to AI because they would fail to ovulate after estrus. These data underscore the importance of implementing a comprehensive reproductive management program for identification and treatment of cows that would otherwise not be inseminated and to identify those cows failing to ovulate when cycling spontaneously. Based on data from this experiment using this accelerometer system (Valenza et al., 2012), the mean time of AI in relation to ovulation was acceptable for most of the cows detected in estrus; however, variability in the duration of estrus and timing of AI in relation to ovulation could lead to poor fertility in some cows.

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