Extension
UNIVERSITY OF WISCONSIN-MADISON

Determining Bovine Pregnancy Status

Originally published 2015, updated and reviewed and updated June 2023

By: Sandy Stuttgen, DVM, Extension Agriculture Educator
Reviewed and updated by Ryan Sterry, Extension Agricultural Educator
Contributions by Heather Schlesser & Ryan Sterry, Extension Agriculture Educators

Maintaining a successful reproductive program in our modern dairy and beef operations requires dedication. While it is rewarding to hear a pronouncement of pregnancy, there is not much the manager can do with that information. Except wait. Wait to recheck later to make sure the pregnancy is progressing. Wait for a calf to be born. So, while it is tempting to focus on a positive pregnancy diagnosis, it is important to remember the real value in pregnancy diagnosis is identifying non-pregnant cows so that action can be taken.

The sooner non-pregnant cows can be identified, the sooner they can be re-inseminated. Coupling a non-pregnancy diagnosis with a management decision to quickly reinitiate AI service improves reproductive efficiency and pregnancy rate. Repeat breeders who fail to become or stay pregnant also offer management the option to make a culling decision.

There are direct and indirect methods used to determine pregnancy status. Direct methods involve the direct detection of the tissues and/or associated fluids of the conceptus either manually by transrectal palpation or visualized by transrectal ultrasound. Indirect methods measure reproductive hormones at specific stages after AI, or the detection of conceptus specific substances in maternal body fluids.

Direct Determinations

A calf's head and feet presenting at birth. An accurate, but time inefficient, method of pregnancy detection. Photo credit: Sandy Stuttgen.

The watch and wait method involves the direct visualization of the calf being born. This method is both sensitive and specific. She either was pregnant and delivered a calf, or she was not; and a calf is not born. While accurate in determining the cow was pregnant, this is the least time sensitive method.

Bumping the calf through the cow's abdominal wall is another direct method of determining pregnancy status. Depending on the size and stature of the cow and the experience of the clinician, the calf may be felt during the last trimester of pregnancy. This is accomplished by vigorously bumping against the cow's right flank with a closed fist to feel the skeletal mass of the calf. Calf bumping is not the best method of non-pregnancy diagnosis; the calf may be small or poorly positioned. Repeated calf bumping on different days at different times may help to improve accuracy of the diagnosis using this method.

Calf "bumping" - pushing on the lower flank. Photo credit: Heather Schlesser.

Beef and Dairy workers should know the signs of estrous behavior. These include vaginal mucous discharge, increased restlessness, and increased vocalization, smelling of other cows, following other cows and attempting to mount them, allowing themselves to be mounted, and a ruffled tail head or mud on their hind flanks from being mounted. Reproductive management plans must include what to do with cows exhibiting estrus.

Return to estrus is perhaps the oldest form of directly determining pregnancy status. The bovine estrous cycle is approximately 21 days long, with a range of 17- 24 days. Absence of estrous activity every three weeks until parturition provides evidence that conception occurred, and the cow did not suffer pregnancy loss. A return to estrus is strong evidence the cow did not conceive, or lost the pregnancy, and the cow is now open. Gestation length in Holsteins is approximately 282 days (9 months).

Cow standing to be mounted during estrus.
Photo credit: Paul Fricke

Successful reproductive managers quickly realize the need for an accurate early determination of pregnancy status so that action can be taken on non-pregnant cows. Managers also need to have confidence in the test result. While the "watch and wait" method is highly accurate, it is the least time sensitive. Considering the average gestation is around 282 days, that's potentially 9 months a cow could be not pregnant, and management doesn't know and can't act upon it. Likewise, estrous detection is an important skill but can have inaccuracies. Failure alone to observe estrus is not enough to confirm pregnancy. There's always the possibility a cow was in estrus, and management failed to observe it.

Palpating or visualizing the developing fetus and placenta is a direct method of determining pregnancy status. Skilled veterinarians performing transrectal palpation can diagnose pregnancy status beginning approximately 35 days post breeding. Accuracy is achieved with experience. The physical attributes of the cow also have an effect. For example, cows with excessive pelvic fat are harder to palpate.

During transrectal palpation, the veterinarian determines a positive pregnancy diagnosis when they palpate a fluid filled uterine horn, the presence of a mature corpus luteum (CL) on the same side (ipsilateral) to the fluid filled horn, presence of amniotic vesicle (approximately 35 days post conception) and/or placenta (approximately 40 days post conception). Palpation also allows evaluation of uterine and ovarian health.

When using transrectal ultrasonography, visualization of a corpus luteum on the ovary ipsilateral to the fluid-filled uterine horn containing an embryo with a heartbeat is the basis for a positive pregnancy diagnosis. This can be confidently visualized 30 days post breeding. Ultrasound allows the visualization of

Veterinarian and portable ultrasound machine. Photo credit: Paul Fricke.

dead embryos, ovarian pathology, uterine morphology, and twins. Fetal sex can be accurately diagnosed at fetal age 55 to 60 days.

Researchers have concluded the overall accuracy of transrectal ultrasonography for determination of pregnancy status 27 days after timed AI to be 95% when the previously determined cows were rechecked. The positive predictive value was 90%. The negative predictive value was 98%. The positive predictive value is lower because of the normal early pregnancy loss; these cows may have been pregnant at the early ultrasound determination, only to be found open at re-check. Twins decrease the positive predictive value of the diagnosis. Pregnancy loss is three-fold higher for cows carrying twins versus singletons (Silva et al.).

How well does the test outcome predict true status of the pregnancy?

Positive predictive value: the test correctly identified those truly pregnant; determination of pregnancy was confirmed with recheck by another method and/or later during the gestation.

Negative predictive value: the test correctly identified those truly not pregnant; determination of non-pregnancy was confirmed with recheck by another method and/or later.

Indirect Determinations

Measuring the reproductive hormone Progesterone is an example of an indirect method. Progesterone (P4) monitoring can be used to identify non-pregnant cows. Cow-side kits which measure P4 in milk are currently available. P4 monitoring has a 95% to 100% negative predictive value, low progesterone levels on day 21, or better yet on day 23 after last estrous (post-AI) indicate the cow is open. Mastitis can disguise progesterone; milk from infected quarters will yield low P4. The positive predictive value of milk P4 is 80%. This low positive predictive value is due to embryonic mortality. P4 will remain high in some cases with uterine infections. (Rhodes).

Because of the need to repeatedly test for P4, it may not be a practical option for beef herds or non-lactating dairy heifers.

The hormone progesterone is secreted by the corpus luteum (CL) and is responsible for maintaining the pregnancy. If cow is pregnant, or a fluid filled uterine horn signals pregnancy, progesterone persists beyond the next heat cycle. At best, you can use milk progesterone tests to tell you if cow is open 21-24 days after last breeding. Monitoring milk progesterone may help you perform better heat detection. Serial monitoring would give you a good indication of her reproductive cycle.

Bovine pregnancy-associated glycoprotein (PAG) is secreted by bi-nucleate cells present in cotyledons. Over 20 different bovine PAGs have been identified. While researchers do not understand the function of these PAGs, current testing techniques allow them to be used as a chemical marker for pregnancy diagnosis.

An ELISA blood test was developed to detect six PAGs as a method for early pregnancy diagnosis. Consistent measurable PAG levels in maternal blood plasma occur 15 days after conception and peak at day 32 post conception. PAG levels then sharply decline to a baseline (nadir) from day 53 to 60 days post conception, gradually increasing to 74 to 102 days. Increasing levels are measured as the cotyledon mass grows while supporting the growing fetus and remains high until 60 days after calving. (Ricci et al, 2015).

First lactation (primiparous) dairy cows have greater PAG levels than second or greater lactation (multiparous) cows. Researchers do not know why the six PAGs measured by ELISA drop to a low level 53 days after conception and then why they rebound. It is unknown what is happening with the other PAGs not measured by the available test.

When commercial blood plasma samples are mailed overnight, it takes approximately 24-36 hours from

sample collection to receive outcomes from the lab. Scheduling with mail services must be considered when submitting samples. On-farm kits for determining plasma PAG levels became available in 2023.

The overall accuracy of determining pregnancy status when measuring plasma PAG at 32 days post AI was found to be 95%. The positive predictive value using plasma PAG was 91%, the negative predictive value was 97%. Prediction of non-pregnancy is highly accurate, just as it is for transrectal ultrasonography. Early pregnancy loss contributes to the lower accuracy of early pregnancy diagnoses (Silva, et.al.)

PAGs secreted in maternal blood are transferred to milk; however, milk PAG levels are approximately two-fold less than plasma PAG levels. Milk PAG levels peak 32 to 39 days post-conception. They then slowly decline to a nadir from 46 to 67, rebounding to higher levels 74 to 102 days post conception. Milk PAG drops sooner and remains lower longer than does plasma PAG. PAGs are measurable in milk for 60 days after calving.

Plasma and milk PAG levels are negatively correlated with milk production for both primiparous and multiparous cows (Ricci et al, 2015). Dilution of milk PAG by increased milk production is not the reason for why milk PAG declines, because plasma PAG also declines as milk production increases. While scientists speculate the reasons for this negative correlation, those evaluating test outcomes must bear in mind the cows' milk production may cause a false negative test result. Rechecks may be needed.

Accuracy of both plasma and milk PAG testing compared to transrectal ultrasonography was evaluated from 25 to 102 days after AI. Overall accuracy for correctly identifying 32-day pregnancy status to recheck status was 92% for plasma and 89% for milk PAG levels. The plasma PAG positive predictive value was 84% while its negative predictive value was 100%. The milk PAG positive predictive value was 78% and its negative predictive value was 99% (Ricci et al).

Drawing a blood sample from the tail vein for PAG testing. Photo credit: Paul Fricke

The Importance of Re-checks:

Pregnancy status should be determined in dairy cows as soon as possible after diagnosis confounded by subsequent pregnancy loss. Various research trials confirm a normal 13% pregnancy loss occurring from 27 to 31 days and 38 to 50 days in gestation (Ricci, et al, 2014). An early diagnosis of pregnancy (less than 50 days after AI) must be re-confirmed later to identify cows that experience early embryonic death.

In Summary:

There are several direct and indirect methods available for determining bovine pregnancy status. Confidence in the result is correlated to the days post service the determination is made. Coupling a non-pregnancy diagnosis with a management decision to quickly re-inseminate the cow improves reproductive efficiency and pregnancy rate.

This chart summarizes the three results of a pregnancy status determination.

Determination Method Used	Result		
	Pregnant	Recheck	Non-Pregnant
Visual return to estrus	Does not return to heat until after calving.	Does not return to heat every 21 days after breeding.	Returns to heat every 21 days.
Bumping the dam's right flank	Fetal skeleton during last trimester.	Fetal skeleton present or absent during last trimester.	Fetal skeleton absent during last trimester.
Transrectal palpation	Fetal skeleton 50-60 days after breeding.	CL, membrane slip/amniotic vesicle 35-40 days after breeding.	No conceptus and no CL 35-40 days after breeding. Developing follicle is present.
Transrectal ultrasound	Fetal sex 58 days after breeding.	Amniotic vesicle/fetal heartbeat 30 days after breeding.	No conceptus and no CL30 days after breeding. Developing follicle is present.
Progesterone assay	High P4 23 days after breeding.	High P4 23 days after breeding.	Low P4 23 days after breeding.
Blood PAG	Positive 74 days after breeding.	Positive day 32 after breeding. False positive <60 days post calving. False negative with high milk production.	Negative 32 days after breeding.
Milk PAG	Positive 74 days after breeding.	Positive day 32-39 after breeding. False positive: < 60 days post calving. False negative with high milk production.	Negative 32-39 days after breeding.

References:

Fricke, P.M., A. Ricci, P. Carvalho, and M Amundson. 2015. Milk vs blood-which is best for PAG pregnancy prediction? Western Dairy Management Conference, Proceedings, Mar3-5, 2015, Reno, NV

Ricci, A., P.D. Carvalho, M.C. Amundson, R.H. Fourdraine, L. Vincenti and P.M. Fricke. 2014. Factors associated with pregnancy-associated glycoprotein (PAG) levels in plasma and milk of Holstein cows during early pregnancy and their effect on the accuracy of pregnancy diagnosis. J. Dairy Sci. 98:2502-2514.

Rhodes, R.C. The use of milk progesterone assays for reproductive management. Retrieved March 3, 2015 from http://www.wvu.edu/~agexten/forglvst/Dairy/dirm9.pdf

Silva, E., R.A. Sterry, D. Kolb, N. Mathialagan, M.F. McGrath, J.M. Ballman and P.M. Fricke. 2007. Accuracy of pregnancy-associated glycoprotein ELISA to determine pregnancy status of lactating dairy cows twenty-seven days after timed artificial insemination. J. Dairy Sci. 90:4612-4622.