A Comparison of Methods for Early Pregnancy Diagnosis

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Early identification of pregnant and nonpregnant cows post breeding improves reproductive efficiency and pregnancy rate in cattle by decreasing the interval between services. Many new and old technologies are available to identify pregnant and nonpregnant animals early post service and can play a key role in an overall reproductive management strategy to rapidly return these animals to the breeding program. This paper reviews rectal palpation, ultrasonography, milk progesterone tests, and the early conception factor (ECF) test for pregnancy diagnosis.

Measure of Accuracy of Pregnancy Diagnosis

Earlier pregnancy diagnosis may go together with increased errors in diagnosis. The accuracy of the diagnosis is expressed by sensitivity and specificity, and positive and negative predictive values. Table 1 explains these terms and gives an example calculation:

Table 1. Calculation and example of sensitivity, specificity, predictive value positive and predictive value negative (100 cows are diagnosed for pregnancy).

		Diagnosis		
		Open	Pregnant	
True status of cow	Open	A = 40 Correct Decision	B = 5 False Negative	A+B = 45
	Pregnant	C = 1 False Positive	D = 54 Correct Decision	C+D = 55
		A+C = 41	B+D = 59	A+B+C+D = 100

Specificity = A/(A+B) = 40/45 = 88.9%
Sensitivity = D/(C+D) = 54/55 = 98.2%
Predictive value negative = A/(A+C) = 40/41 = 97.6%
Predictive value positive = D/(B+D) = 54/59= 91.5%

Specificity is the probability that a cow is diagnosed to be open, given that the cow is truly open: A/(A+B) = 40/45 = 88.9%. Sensitivity is the probability that the diagnosis is pregnant, given that cow is truly pregnant: D/(C+D) = 54/55 = 98.2%. The predictive value negative is the probability that the cow is truly open, if the diagnosis is open: A/(A+C) = 40/41 = 97.6%. Predictive value positive is the probability that the cow

is truly pregnant if the diagnosis is pregnant: D/(B+D) = 54/59 = 91.5%. Pregnancy diagnosis should have a high predictive value negative and high sensitivity.

Rectal Palpation

Palpation of the uterine contents rectally is probably the most commonly used method for pregnancy diagnosis. Pregnancy diagnosis after insemination can be conducted as early as 30 days in heifers and 35 days in cows, although much practice is necessary in order to determine pregnancy at that stage. Several palpable structures are indicative of pregnancy. Due to accumulation of fluids within the pregnant uterine horn, one of the initial signs of pregnancy is a difference in size of uterine horns (uterine asymmetry). Also, it is possible to feel the slipping of the fetal membrane along the greater curvature within the uterus (membrane/fetal slip). There is a rule of thumb that is quite useful in estimating fetal age based on the size of the fetus in relationship to the size of some well known animals. This rule of thumb is detailed in the following Table 2.

Table 2. Calf fetal size at various stages of pregnancy in relation to the size of some commonly known adult animals. (source: P.J. Hansen)

Stage of pregnancy	Calf fetal size in relation to the size of commonly known adult animals
2 months	mouse
3 months	rat
4 months	small cat
5 months	large cat
6 months	beagle dog

Table 3 presents the uterine position and diameter, as well as structures felt at palpation according to stage of pregnancy. The following describes when specific structures can first be palpated: Membrane Slip - 30-35 days, Amniotic vesicle - 35-60 days and the Fetus – 65+ days.

Rectal palpation has the advantage of being an accurate, fast, relatively cheap method that is less labor intensive as compared to the previous methods. Nonetheless, training is necessary and the exam should be conducted by a veterinarian or by an experienced herdsman. The main disadvantage of rectal palpation is that it cannot be performed until later in gestation than some other methods. Some veterinarians are able to determine pregnancy by palpation as early as 35 days after insemination, but usually rectal examinations take place between 45 and 60 days after insemination to increase the accuracy of the exam.

Table 3. Uterine position/diameter and structures during pregnancy. (source: P.J. Hansen)

Stage of pregnancy (days of gestation)	Uterine position	Uterine diameter	Palpable Structures
35-40	Pelvic floor	Slightly enlarged	Uterine asymmetry/fetal slip
45-50	Pelvic floor	5.0 - 6.5 cm	Uterine asymmetry/fetal slip
60	Pelvis/abdomen	6.5 - 7.0 cm	Membrane slip
90	Abdomen	8.0 - 10.0 cm	Small placentomes/fetus (10-15 cm long)
120	Abdomen	12 cm	Placentomes/fetus (25-30 cm long)/fremitis
150	Abdomen	18 cm	Placentomes/fetus (35-40 cm long)/fremitis

Ultrasonography

In the 1980s, real time ultrasonography was developed for use in domestic animals. An ultrasound machine resembles a radar device. A probe is inserted through the rectum and positioned above the uterus. Real-time, B-mode ultrasound is a display mode in which the signal echoed from a tissue is displayed as a dot. The intensity of the dot is proportional to the amplitude of the signal and its position is relative to the distance between the probe and the reflective tissue. The image is 2-dimensional, created by rapid succession of B-mode traces so that as the ultrasound probe moves, the image changes, depicting motion in real-time. The ultrasound probe acts to send and receive sound waves. When an electric field is applied to the crystals in the probe, they change shape and vibrate like cymbals, creating waves of sound. The ultrasound probe directs these high frequencies, low intensity sound waves toward the tissues. Different proportions of the sound waves emitted are reflected back to the probe, depending on the density of the tissue. The returning sound waves produce pressure on the crystals, generating an electric charge, which is converted to a visual image on the screen. Fluid, such as blood or follicular fluids, does not reflect sound waves and no image (black) appears on the screen. Bone is the densest tissue and reflects sound waves almost completely depicting white images. Other tissues reflect varying proportions of sound waves and produce images

Figure 1. Ultrasound of a 25-Day-old fetus.

of various shades of gray. The differences in the reflection of sound waves from various tissues or differences in the angle at which sound waves strike tissue surfaces may cause echoes.

Real-time, B-mode ultrasonography has been reported to detect pregnancy in cattle as early as 9 (Boyd et al., 1988) or 12 days (Pierson and Ginther, 1984) into gestation. Other reports, however, have disputed those claims and emphasized that the accuracy of ultrasound diagnosis of pregnancy on Day 10 through 16 is not significantly better than a random guess (<50%). Accuracy of diagnoses improves, however, by Day 18 (85%), 20 (100%) and 22 (100%) of pregnancy (Kastelic et al., 1989). Presence and vitality of the embryo can be confirmed by the detection of a heartbeat as early as 19 to 24 days of gestation. The embryo initially appears as a short line (Day 20-22), later becomes C-shaped (Day 22-30), and finally, by Day 30-32 of gestation assumes an L shape. Although the embryo can first be detected between Days 19 and 24 of gestation (Curran et al., 1986), it is most practical to scan females which are expected to have embryos that are >26 days of age.

The main advantages of the use of ultrasound for pregnancy diagnosis are 1) the high accuracy of the results that are generated and 2) the fact that pregnancy diagnosis may be conducted relatively early after insemination. The main disadvantages of the use of ultrasonography are related to cost and time involved with the use of this technique. Ultrasound machines are relatively expensive and it takes more time to perform a pregnancy diagnosis with an ultrasound machine than by rectal palpation. Also the training of the operator needed to interpret the images also can serve as a disadvantage. Cows diagnosed pregnant at an early ultrasound exam have a greater risk of early embryonic loss and, therefore, must undergo subsequent pregnancy examinations to identify and rebreed cows that experience such loss. If left unidentified, cows experiencing embryonic loss after an early pregnancy diagnosis would actually reduce reproductive efficiency by extending their calving interval (Fricke, 2002).

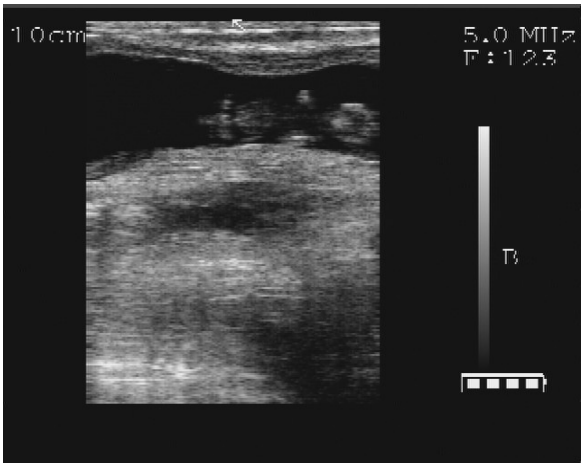

Figure 2. Ultrasound of a 50-Day-old fetus.

Milk Progesterone Tests

Progesterone is the hormone also referred to as the pregnancy hormone. The progesterone test is based on the expected changes in the production of this hormone

during the reproductive cycles and pregnancy. Progesterone levels elevate during the midcycle of each reproductive cycle and during the entire gestation period. Since progesterone is produced by the corpus luteum in the ovary, high progesterone levels show a functional corpus luteum. The corpus luteum forms and produces high amounts of progesterone after the cow has been in estrus and ovulated.

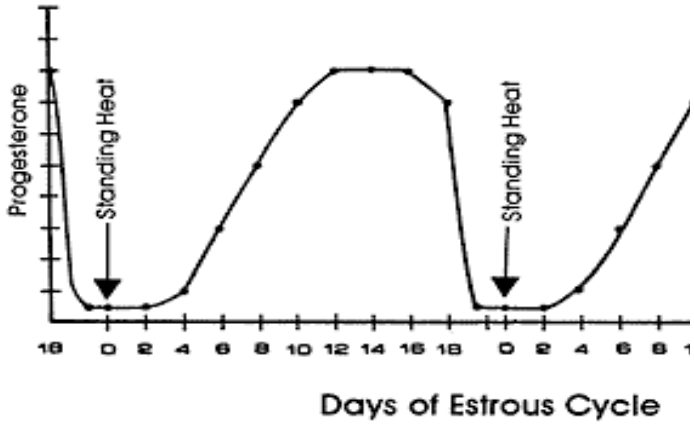

Figure 3. Progesterone concentrations in milk reoccurring estrous cycles (source: Larson, 1997)

If the cow is not pregnant, the corpus luteum regresses and progesterone levels decline to low levels about 2 days before the cow comes into heat again. However, if the cow becomes pregnant, the corpus luteum continues to function and progesterone levels remain high throughout gestation. Therefore, if the cow is not pregnant and has regular estrous cycles, progesterone levels in the milk follow a cyclic pattern being low from about 2 days before heat until about 4 to 5 days after heat and high during the middle portion of the cycle. Since progesterone is essential for the maintenance of pregnancy, it is continuously high in pregnant cows.

To use the milk progesterone test as a pregnancy indicator the milk sample must be collected between 21 to 24 days after the cow was in estrus and inseminated. Low progesterone would indicate that the cow is not pregnant and high progesterone would indicate that the cow has a functional corpus luteum and might be pregnant. Therefore, the test is most accurate in determining that a cow is not pregnant, because if the progesterone levels are low she cannot be pregnant.

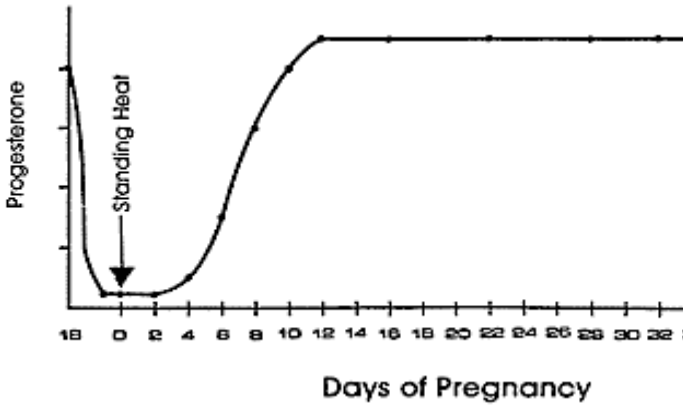

Figure 4. Progesterone concentrations in milk remain high throughout pregnancy (source: Larson, 1997).

Reasons milk progesterone levels might be high between 21 and 24 days after insemination include:

- The cow is pregnant.
- The cow is in the middle of her estrous cycle but not pregnant due to:

    - an error in estrous detection and the cow was bred at wrong time
    - a longer than usual estrous cycle.
- Embryonic mortality. The cow conceived but the embryo died.
- Abnormalities, such as pyometra (accumulation of pus in the uterus or a dead, mummified fetus).

Numerous studies indicate that the accuracy of early pregnancy diagnosis by milk progesterone is only about 80%. Reasons for this include: variation in estrus cycle length between cows, estrus detection errors, uterine disease (pyometra), ovarian dysfunction (luteal or follicular cysts), and early embryonic mortality, to name a few. In short, the reliability of milk progesterone for the diagnosis of pregnancy is not satisfactory in it and should be confirmed by palpation or ultrasound. However, with a series of samples taken at day 0 (the date of insemination) and days 21 and 24 the accuracy of making an early diagnosis of non-pregnancy approaches 95 to 100% (Nebel et al., 1988). Therefore, the milk progesterone test is a tool in the determination of the non-pregnant cow. The advantage of this early confirmation of non-pregnancy prevents the further loss of early breeding opportunities. Cowside milk progesterone assays conducted between 18 and 24 days post AI had an overall reported accuracy of 97.2% for cows identified as nonpregnant (Pennington et al., 1985), representing the earliest proven method for identifying nonpregnant animals.

The Early Conception Factor (ECF) Test

Recently, a new early pregnancy test has become commercially available for use in cattle. The Early Conception Factor (ECF) test (Concepto Diagnostics, Knoxville, TN) reportedly detects a pregnancy-associated glycoprotein within 48 hours of conception. Two studies have compared results from the ECF test conducted between Days 3 to 7 and Days 11 to 15 post-AI to pregnancy diagnosis using palpation per rectum and ultrasound ranging from 25 to 60 post-AI (Adams and Jardon, 1999; DesCôteaux et al., 2000). Results for test sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 86%, 4%, 49%, 23%, and 46%, respectively. Although the observed agreement between readers (91% for Test 1; 89% for Test 2) and between tests for the same serum sample (94% for Reader 1; 91% for Reader 2) was high, the overall rates of false positive and false negative ECF test results were 96% and 14%, respectively. Cordoba et al. (2001) concluded that the ECF test, in its present form, is an unreliable method for determining pregnancy status on day six after estrus in dairy cattle. Although the predictive value of a positive ECF test result increases as the conception rate in a herd increases, the predictive value of a negative ECF test result would be less than 50% in dairy herds exhibiting a conception rate greater than 25%.

Summary

Dairy Producers need to find a technique or combination of techniques that will deliver the level of accuracy in pregnancy diagnosis they desire within the confines of availability of experienced labor and their current facilities. Table 4 briefly summaries the strengths and weaknesses of each technique.

Table 4. Comparison of early pregnancy diagnosis techniques.

Pregnancy Diagnosis Technique	Early Testing Time	Diagnosis Pregnancy Accurately	Diagnosis Non-Pregnancy Accurately
Rectal Palpation	♦	♦♦♦	♦♦♦♦
Ultrasound	♦♦	♦♦♦♦	♦♦♦♦
Milk Progesterone	♦♦♦	♦♦	♦♦♦
ECF	♦♦♦♦	♦	♦

Literature Cited

1. Adams, C.S., and P.W. Jardon. 1999. Evaluation of the early conception factor tests in cows 3-7 days post-breeding. Proc. Am. Assoc. Bov. Pract. 32: 240-241.
2. Beal, W.E., R.B. Edwards, III and J.M. Kearnan. 1989. Use of B-mode, linear array ultrasonography for evaluating the technique of bovine artificial insemination. J. Dairy Sci. 72:2198.
3. Boyd, J.S., S.N. Omran and T.R. Ayliffe. 1988. Use of a high frequency transducer with real time B-mode ultrasound scanning to identify early pregnancy in cows. Vet. Rec. 123:8.
4. Cordoba, M.C., R. Sartori, and P.M. Fricke. 2001. Assessment of a commercially available early conception factor (ECF) test for determining pregnancy status of dairy cattle. J. Dairy Sci. 84:1884.
5. Curran, S., R.A. Pierson and O.J. Ginther. 1986. Ultrasonographic appearance of the bovine conceptus from days 20 through 60. J. Amer. Vet. Med. Assoc. 189:1295.
6. DesCôteaux, L., P.D. Carrière, and M. Bigras-Poulin. 2000. Evaluation of the Early Conception Factor (ECF) dipstick test in dairy cows between days 11 and 15 post-breeding. Bov. Pract. 34:87-91.
7. Fricke, P. M. 2002. Scanning the future-ultrasonography as a reproductive management tool for dairy cattle. J. Dairy Sci. 85:1918.
8. Kastelic, J.P., S. Curran, and O.J. Ginther. 1989. Accuracy of ultrasonography for pregnancy diagnosis on days 10 to 22 in heifers. Theriogenology 31:813.
9. Nebel R.L. 1998. On-farm progesterone tests. J. Dairy Sci. 71:1682-1690.
10. Pennington, J.A., L.H. Schultz, and W.F. Hoffman. 1985. Comparison of pregnancy diagnosis by milk progesterone on day 21 and day 24 postbreeding: field study in dairy cattle. J. Dairy Sci. 68:2740–2745.

11. Pierson, R.A. and O.J. Ginther. 1984. Ultrasonography for detection of pregnancy and study of embryonic development in heifers. Theriogenology 22:225.
12. Ruiz, F.J., Oltenacu, P.A., Smith, R.D. 1989. Evaluation of on-farm milk progesterone tests to determine non pregnant cows and to prevent insemination errors. J. Dairy Sci. 72:2718-2727.

Notes