

Pregnancy Determination in Cattle: a Review of Available Alternatives

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Introduction

Pregnancy diagnosis in cattle is a widely practiced procedure and has come to be accepted as a highly recommended management technique in beef cow herds. Pregnancy detection offers the following potential benefits in cow/calf management programs:

- Timely culling of open cows
- Aid in the culling decision for cows with other issues
- Breeding management (move to next calving season, for example)
- Grouping cows for feeding, calving, other management

Potential economic benefits of pregnancy detection include timely culling, saving costs maintaining cows which will not provide economic returns and providing information to allow planning for replacement needs. As many cows in US herds calve in the spring, fall pregnancy checking allows culling open cows to avoid wintering costs. In many settings, wintering costs may constitute half the annual cost of a cow. Culling open cows before winter saves the cost of wintering a cow that will provide no return. Figure 1 shows the percentage of operations in the US, as reported by NAHMs that use the pregnancy status of cows in culling decisions. Note that only age is used more often used and that the larger an operation (and probably more economically motivated), the more likely they are to use an open or late calving diagnosis in a culling decision.

Staging of pregnancy as a part of pregnancy checking also has potential economic advantages. Knowing if cows will calve early or late in the calving season may influence the culling decision for early or late calving cows that have other issues. In some cases cows may be grouped for different management (feeding, housing, calving watches, etc.) based on their stage of pregnancy at pregnancy diagnosis.

Current methods for pregnancy diagnosis

Currently US cattle producers have available to them three different methods available for pregnancy diagnosis:

- Examination by rectal palpation of the reproductive tract
- Examination by transrectal real-time, linear array ultrasonography
- Blood testing based on the determination of one or the other of two placental source substances present in the blood of pregnant cows

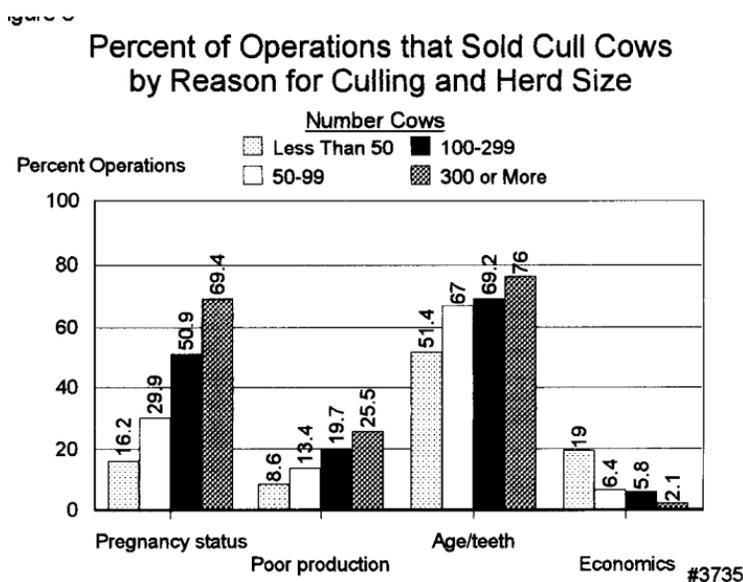


Figure 1. Beef '97 Part I: [Reference of 1997 Beef Cow-Calf Management Practices](http://www.aphis.usda.gov/animal_health/nahms/beefcowcalf/#beef97) , [Culling Practices](http://www.aphis.usda.gov/animal_health/nahms/beefcowcalf/#beef97) in Beef Cow-Calf Operations
http://www.aphis.usda.gov/animal_health/nahms/beefcowcalf/#beef97

Rectal Palpation for Pregnancy

Rectal examination of cows is a very time-honored technique for the diagnosis of pregnancy. The proximity of the reproductive tract of the cow to the rectum and its elasticity allows a trained operator to detect characteristics of the tract that coincide with either pregnancy or nonpregnancy. Four cardinal signs of pregnancy have been determined to be very reliable determinants of pregnancy, namely: 1) the displacement of the chorio-allantoic membrane as differential pressure is applied to the uterine horn (called the fetal membrane “slip”); 2) palpation of the amniotic vesicle which has enough turgor to be recognizable until day 65 to 70 of pregnancy; 3) palpation of the fetus which can be detected as soon as the amniotic vesicle loses its turgor at about 70 days of gestation ; and 4) palpation of the placentomes (cotyledon and associated caruncle) in the wall of the pregnant uterus. Non-pregnancy is determined by a thorough examination of the uterus, usually after it is retracted onto the floor of the pelvis and the absence of the cardinal signs of pregnancy is carefully ascertained.

Rectal palpation also allows an estimation of the stage of pregnancy. Table 1 below shows characteristics of pregnancies at various stages of gestation. Kasimanickam et al [Clin Therio 2(4): 2010] reported on the accuracy of prediction of pregnancy length estimation: “The pregnancy status was diagnosed and days in gestation of each cow were determined by per-rectal palpation 70 d after AI. Projected gestation length was determined based on days of gestation at pregnancy diagnosis and projected calving date. The gestation length (281.2 ± 4.71 d; Mean ± SD) for the study population was retrospectively calculated from breeding and calving dates. These projected and actual gestation lengths were matched to determine the error in diagnosing pregnancies resulting from AI or by natural service. The error in pregnancy diagnosis was classified as error due to over-estimation (diagnosed pregnant to AI but pregnant to bulls) or

under-estimation (diagnosed pregnant to bulls but pregnant to AI) using mean ± 2 SD as cut-off. The error rate for pregnancy diagnosis was estimated as 7.0 % (3.4% due to under-estimation and 3.6% due to over-estimation) in differentiating AI pregnancy from bull-breeding pregnancy for the gestation length of 281 days. “Very experienced clinicians in this study had error rates as low as 3.0%.

Table 1. Fetal Size and Characteristics Used in Determining Pregnancy.				
Stage	Days of Gestation	Fetal Size		Identifying Characteristics
		Weight	Length Inches	
I	30	1/100 oz.	2/5	One uterine horn slightly enlarged and thin; embryonic vesicle size of small marble. Uterus in approximate position of nonpregnant uterus. Fetal membranes may be slipped between fingers from 30 to 90 days.
	45	1/8- 1/4 oz.	1-1 ¼	Uterine horn somewhat enlarged, thinner walled and prominent. Embryonic vesicle size of small egg.
	60	¼ - ½ oz.	2 ½	Uterine horn 2 ½ to 3 ½" in diameter; fluid filled. Fetus size of mouse.
	90	3-6 oz.	5-6	Both uterine horns swollen (4 to 5" in diameter). Fetus is size of rat. Uterine artery 1/8 to 3/16" in diameter. Cotyledons ¾ to 1" across, but very soft.
II	120	1-2 lb.	10-12	Similar to 90-day but fetus more easily palpated. Fetus is size of small cat with head the size of a lemon. Uterine artery 1/4" in diameter. more noticeable and 1 ½ inches in length. Horns are 5 to 7" in diameter.
	150	4-6 lb.	12-16	Difficult to palpate fetus. Uterine horns are deep body cavity with fetus size of large cat – horns 6" in diameter. Uterine artery ¼ to 3/8" in diameter. Cotyledons 2 to 2 ½" in diameter.
III	180	10-16 lb.	20-24	Horns with fetus still out of reach. Fetus size of dog. Uterine artery 3/8 to 1/2" in diameter. Cotyledons more enlarged. From sixth month until a movement of fetus may be elicited by grasping feet, legs or nose.
	210 240 270	20-30 lb. 40-60 lb. 60-100 lb.	24-32 28-36 28-38	From 7 months until parturition fetus may be felt. Age is largely determined by increase in fetal size. The uterine artery continues to increase in size- 210 days, 1/2" in diameter; 240 days, 1/2 to 5/8" diameter; 270 days, ½ to ¾" in diameter.

Ultrasonography for Pregnancy Diagnosis

Ultrasonography has become practical for routine pregnancy diagnosis in cattle as machines which are portable, durable and affordable have become available to veterinarians and technicians. A probe that emits the ultrasound waves (5.0 MHz or 7.5MHz) is inserted into the rectum of the cow. The ultrasound waves bounce differently off of fluids or different solid tissues and this difference in waves returning (called echogenicity) is sensed by the probe that then sends signals to the electronics of the machine. These signals are then translated into a picture that is displayed on a CRT, flat screen or other visual device such as a small screen in a set of goggles. The ability to actually “see” the reproductive tract, fluids of pregnancy and fetal parts results. Some pregnancy associated structures can be seen as early as 9 days after conception. However, reliable pregnancy detection is generally accepted after 26 to 30 days. At this stage a fetal heart beat can usually be seen assuring the viability of the fetus.

The early confidence in pregnancy detection using transrectal ultrasonography has led to the implementation of “resynchronization” programs, especially in dairy cattle reproduction. Cows are often administered reproductive hormones after a diagnosis of nonpregnant that allow cows to be rebred very quickly. Of course, the administration of Prostaglandin F2a results in abortion if pregnancy is present so a very accurate diagnosis is crucial.

Since the fetus can be visualized, gender determination can be performed. Successful fetal sexing has been reported from 50 to 120 days (Lamb, G.C., and P.M. Fricke. 2004. ARSBC. North Platte, NE. pp 219-229). The genital tubercles (which become the vulva or the prepuce) have a different appearance and are in different locations for the male vs. the female fetus by day 50. Eventually the scrotum, vulva and teats can be seen. Knowing the gender of a fetus may aid in culling, selection and sale decisions.

Blood tests for pregnancy diagnosis

In the 1980's proteins were discovered in the blood of pregnant cows that are produced by the conceptus. The first of these proteins to be developed into a commercial test for pregnancy is called “Pregnancy Specific Protein B” or PSPB. This protein is now known to be produced by the placenta. Other products produced by the conceptus and present in the blood soon after pregnancy begins are a group of compounds called “Protein Associated Glycoproteins” or PAGs. The substance called Protein Associated Glycoprotein 1 or PAG1 can now be detected by a commercial test.

These pregnancy associated compound can be detected in the blood of some cows in the first couple of weeks of pregnancy. However, development of practical tests means dealing with systems that are functional commercially and individual cow variation. In the end, the commercial tests now available claim reliable pregnancy detection at 28 or 29 days after conception.

The original detection of PSPB and PAG1 was done using a technique called radioimmuno assay (RIA). While this technique can be very sensitive and accurate, the use of radioactive materials limits its commercial application. A diagnostic technique called Enzyme-Linked ImmunoSorbent Assay (ELISA) has been developed and used in a host of scientific settings. This technique is used in the currently available early pregnancy tests that detect both PSPB and PAG1.

Blood tests for pregnancy in cattle in the US have been available commercially since 1992. Two companies currently market the tests in the US. Until recently both tests have been laboratory tests. Blood samples for these laboratory tests are taken in the field, mailed to a laboratory and results from the test are returned in a few days. Same day service has recently become available. Also newly available are field tests that can be done at, for example, a veterinary clinic. None claims to be a “cowside” test that can be done at the farm while cattle are still confined for blood collection. Milk tests are being used in the dairy industry and are being integrated into milk sample collection used in ongoing herd record analysis programs.

One of the challenges to use of the pregnancy protein blood tests is inherent in the time after a pregnancy terminates that the substances persist in the cow’s blood. Depending on the test, cows that have recently calved must be 60 to 73 days after calving so that the substances from the prior pregnancy don’t give a false diagnosis of pregnancy. Early losses of pregnancy may also give a false positive test result. One company reports an approximate 5 percent false positive rate due to pregnancies that are lost soon after the test is made or had already been lost when the blood sample was taken.

Comparison of pregnancy tests

Table 2 summarizes thirteen studies, published over 35 years assessing the safety and accuracy of the three major methods of pregnancy detection available to producers today. One of the challenges of any study that attempts to measure the accuracy of a pregnancy detection method is the measure to which the technique is compared. Even if calving rates are used for comparison, pregnancy loss between the time of pregnancy diagnosis and calving certainly complicate the comparison. In fact, distinguishing pregnancy loss not associated with the diagnostic technique is the inherent challenge in any pregnancy diagnosis trial.

An examination of the thirteen studies shows that none of the three methods for pregnancy detection, rectal palpation, rectal ultrasound nor the blood tests is one hundred percent accurate. Every study reported cows that were diagnosed pregnant that did not calve and, if followed, cows that were determined to be open on the initial examinations that were later determined to have been pregnant. It also does not become clear from an examination of the studies that one method is singularly superior to the others.

The safety of rectal palpation for the pregnancy has also been a source of concern for many years. Study number 2 in the table has especially been cited as documenting fetal loss associated with palpation. This study compared the palpation for: 1) fluid only; with 2) palpation for fluid and an amniotic vesicle; with 3) palpation for fluid and the performance of a membrane slip. The study offered no other proof for the diagnosis of the original pregnancy than the diagnosis made by the examiner so that misdiagnosis (calling an open cow pregnant) could not be distinguished from a pregnancy damaged by palpation. The significant differences in pregnancy loss to calving reported were between membrane slip and palpation for fluid only. Much of this loss was attributed to one of three palpators whose losses were significantly greater than the other two palpators. It is interesting to note that the study reported that cows that were palpated by veterinary students were no more likely to lose pregnancies than were cows examined only by experienced clinicians.

With the advent of ultrasound diagnosis of pregnancy it has now become possible to compare losses after rectal palpation with those detected with ultrasound. Study 13 by Romano et al was published in 2013. All 928 cows in the study were diagnosed pregnant with ultrasound at 31 days of gestation. About half of the cows were controls that were not subjected to manual examination of the uterus. About one quarter of the cows were palpated using a single membrane slip technique. The final quarter of the cows was palpated using a double membrane slip technique. Cows were reexamined with ultrasound at days 45 and 60 to ascertain if pregnancy loss had occurred. It was reported that palpation in early gestation did not increase pregnancy loss.

In summary, this report submits the following as a comparison of the strengths and weaknesses for the three major approaches to pregnancy determination that are now available to cattle producers for the diagnosis of pregnant and nonpregnancy in beef cattle.

Advantages of rectal palpation pregnancy diagnosis:

- Immediate determination (usually): Allows sorting, separation, assessment on the spot
- Little equipment needed
- Staging of pregnancy
- Some assessment of viability
- Good to fair assessment of normality of fetus, dam
- Cheap to moderate cost

Disadvantages of rectal palpation:

- Invasive
- Long learning curve
- Potential for damage to dam, fetus ?
- Availability of veterinarian. Technician
- Potential for error

Advantages of ultrasound:

- Immediate determination (usually): Allows sorting, separation, assessment on the spot
- Staging of pregnancy
- Excellent assessment of viability
- Good assessment of normality of fetus, dam

Disadvantages of ultrasound:

- Invasive
- Expensive equipment needed
- Long learning curve
- Potential for damage to dam?
- Availability of veterinarian. Technician
- Potential for error
- Moderate to high cost
- Increased time?

Advantages of blood test:

- Non-Invasive
- Less skill needed
- Available when no technician/ veterinarian available
- Relatively inexpensive equipment
- Shorter learning curve
- Less potential for error? (time after calving restraints, record keeping)

Disadvantages of blood test:

- Moderate to high cost
- No immediate determination : Doesn't allow sorting, separation, assessment on the spot ("Same-day" quick test helps)
- No staging of pregnancy
- No assessment of viability
- No assessment of normality of fetus, dam

Table 2. Major research reports evaluating the safety and accuracy of different methods for the detection of pregnancy in cattle

Study No.	Authors	Title	Reference	Measured	# of Cows	Control	Conclusion
1	Larry G. Paisley, W. Duane Mickelsen, O. L. Frost	A survey of the incidence of prenatal mortality in cattle following pregnancy diagnosis by rectal palpation	Theriogenology 9, no. 6 June 1978 481-491	Cows were checked by rectal palpation at less than 35 d, 35-45 d, and GT 45 d. Followed to calving. No controls.	802 pregnancies in dairy cows	None	The incidence of prenatal mortality in pregnancies diagnosed at less than 35 days, between 35 and 45 days and more than 45 days was 5.8%, 6.0% and 0.8% respectively. When pregnancies that were considered abnormal at the time of diagnosis, abortions occurring more than 100 days after palpation and twin abortions were excluded the incidences were 1.94%, 1.77% and 0% for the three groups respectively. Multiple palpations may have contributed to the losses reported.
2	Abbitt, B., Ball, L., Kitto, G.P., Sitzman, C.G., Wilgenburg, B., Raim, L.W., Seidel Jr., G.E.	Effect of three methods of palpation for pregnancy diagnosis per rectum on embryonic and fetal attrition in cows	Journal of the American Veterinary Medical Association Volume 173, Issue 8, 1978, Pages 973-977	Cows in 2 trials checked by vets or vet student (trial2) by rectal palpation at days 35 to 70. Used fluid only, fluid plus membrane slip or fluid plus amniotic vesicle.	1350 pregnancies in dairy cows	None	Trial 1: no difference. Trial 2 cows had more loss before either a later palpation or at calving for cows checked with a membrane slip than with fluid palpation only. One clinician had significantly more membrane slip losses than other two (accounted for more losses due to membrane slip overall?). No difference between experienced clinicians only and clinicians plus students.
3	R. G. Sasser et. Al	Detection of Pregnancy by Radioimmunoassay of a Novel Pregnancy-Specific Protein in Serum of Cows and a Profile of Serum Concentrations during Gestation	BIOLOGY OF REPRODUCTION 35, 936-942 (1986)	Rectal Palpation vs. blood test at 27 to 102 days then recheck at 77 to 152. Slaughtered apparent "open" cows	102 cows	Slaughter of open cows	Rectal palpation unable to detect 12 early pregnant cows on first exam. Doc called an 88 day pregnant cow open

Study No.	Authors	Title	Reference	Measured	# of Cows	Control	Conclusion
4	P. Humbolt, R. G. Sasser	Pregnancy-specific protein B, progesterone concentrations and embryonic mortality during early pregnancy in dairy cows	J. Reprod. Fert. (1988) 83, 215-223	Apparent Late Embryonic loss	147 cows and heifers	Rectal palpation at 70 to 79 days	Only 30% of pregnant females with extended luteal function (late embryonic mortality) have detectable PSPB levels which are lower than in pregnant cows;
5	B.M. Alexander et al	Embryonic loss from 30 to 60 days post breeding and the effect of palpation per rectum on pregnancy	<u>Theriogenology</u> <u>Volume 43, Issue 3,</u> <u>February 1995, Pages</u> <u>551-556</u>	Pregnancy loss after a single palpation between 30 and 45 days	1359 Holstein Heifers	Second blood test	These findings establish that there was substantial loss of embryos between 30 and 60 d post breeding (18.4%) but that embryo loss was not affected by palpation per rectum.
6	O Szenci ¹ , P. Humblot, R Sasser et al	Comparison of ultrasonography, bovine pregnancy-specific protein B, and bovine pregnancy-associated glycoprotein 1 tests for pregnancy detection in dairy cows	<u>Theriogenology</u> <u>Volume 50, Issue 1, 1</u> <u>July 1998, Pages 77-</u> <u>88</u>	2 ultrasound exams from days 26 to 58 vs. 2 different blood tests	138 dairy cows	Calving rates	Late embryonic mortality in 8.6% of cows. When compared with the noncalving cows, significantly fewer (P<0.001) false positive diagnoses were made by the 2 ultrasonographic tests than by the PSPB and bPAG 1 tests, while significantly fewer (P<0.001) false positive diagnoses were made by the bPSPB test than by the bPAG 1 test. The accuracy of detecting nonpregnant animals by both protein tests was limited by the relatively long half-life of these proteins after calving and by early embryonic mortality.
7	O Szenci et al	Evaluation of false ultrasonographic diagnoses in cows by measuring plasma levels of bovine pregnancy-associated glycoprotein 1	<u>Veterinary</u> <u>Record 1998,142:304-</u> <u>306</u>	Ultrasound vs. bPAG1 blood test	189 ultrasound exams on 56 dairy cows at days 27-31, 34-38, 41- 45 and 55- 59	Calving rates	The results showed that before day 31, ultrasonographic scanning was not very sensitive because six of the 30 calving cows were incorrectly diagnosed as non-pregnant.

Study No.	Authors	Title	Reference	Measured	# of Cows	Control	Conclusion
8	DP NATION, J MALMO, GM DAVIS and KL MACMILLAN	Accuracy of bovine pregnancy detection using transrectal ultrasonography at 28 to 35 days after insemination	Aust Vet J Vol 81, Nos 1 & 2, January, February 2003	Cows were examined by transrectal ultrasonography from 28 to 35 days after insemination. When pregnancy was not confirmed by the observation of a fetus, a second examination 7 days later, confirmed these remaining cows as pregnant or not pregnant to the first insemination.	480 dairy cows	Detection of pregnancy at this early examination was compared with manual transrectal pregnancy examination performed at 13 weeks.	13 of 480 checks were called negative but later pregnant. Fetal loss between the early detection and the 13-week examination (9% of pregnancies) indicated that 28 to 35 days post insemination was too early to reliably detect pregnancy.
9	J.E. Romano and Larson	EARLY PREGNANCY DIAGNOSIS AND EMBRYO/FETUS MORTALITY IN CATTLE	Texas A&M Doctoral Thesis	Difference in pregnancy loss between ultrasound only (50 % of females) and ultrasound plus rectal palpation	1400 cow and heifer ultrasound exams	Ultrasound	Palpation per rectum using the fetal membrane slip for pregnancy diagnosis did not increase embryo/fetus mortality when compared with a positive control group of non-palpated females.
10	M. W. Breed et al	Comparison of pregnancy diagnosis in dairy cattle by use of a commercial ELISA and palpation per rectum	<u>Journal of the American Veterinary Medical Association August 1, 2009, Vol. 235, No. 3, Pages 292-298</u>	Pregnancy difference between palpation between 35 and 75 days and blood test (PSBP) collected the same day (Gp1) or 7 d prior (Gp2)	1483 dairy cows	Rectal palpation at 70 to 79 days	Gp 1 (5%) were nonpregnant by palpation per rectum. Gp 2 (12%) were nonpregnant by palpation per rectum compared to PSBP blood test. Conclusions and Clinical Relevance—Good agreement existed between the 2 tests, especially at longer intervals after insemination. Discrepant results appeared to be attributable to a nonviable fetus, embryonic loss, or fetal loss.

Study No.	Authors	Title	Reference	Measured	# of Cows	Control	Conclusion
11	J.E. Romano and Larson	Accuracy of pregnancy specific protein-B test for early pregnancy diagnosis in dairy cattle	Theriogenology Volume 74, Issue 6, 1 October 2010, Pages 932-939	Disagreement between ultrasound and blood test (PSPB) both done on days 28, 30 and 35. Some tests classified as "probable" or "unlikely"	738 dairy cows	Ultrasound	At Days 28, 30, and 35, percentages of uncertain samples were 8.5, 4.8, and 3.3%, respectively (P < 0.01), and Kappa values were 0.92, 0.92, and 0.95. False negative and false positive results were attributed to low concentrations of PSP-B in pregnant animals and to persistence of pregnant concentrations of PSP-B in females with pregnancy loss, respectively. In conclusion, PSP-B ELISA was a sensitive, specific, and accurate test for pregnancy diagnosis (relative to ultrasound) at Days 28, 30, and 35 after breeding.
12	R. D. Richardson,*1 R. G. Mortimer,† and J. C. Whittier	Comparison of Fetal Losses from Diagnosis of Pregnancy Using Ultrasonography or Rectal Palpation in Beef Heifers by Novice or Experienced Technicians	The Professional Animal Scientist 26 (2010):341-346	Difference in pregnancy loss between heifers preg checked 42 to 74 d by students, interns or experienced technicians by ultrasound or rectal palpation. Some heifers checked by students only!	2190 beef heifers	Recheck by rectal palpation by experienced technician	Overall loss was 1.55%. Risk of loss was greater (P < 0.01) in heifers <53 d pregnant compared with heifers ≥53 d (3.46 vs. 1.26%); a 2.74-fold increase at the time of evaluation. Greater fetal loss (P = 0.051) occurred with rectal palpation than with ultrasonography (2.68 vs. 1.29%; a 2.08-fold increase). Heifers evaluated by inexperienced technicians had a 2.07% fetal loss, whereas heifers evaluated by experienced technicians had only a 1.06% loss (P < 0.01; a 1.95-fold difference).
13	J.E. Romano et al	Effects of early pregnancy diagnosis by palpation per rectum on pregnancy loss in dairy cattle	JAVMA, Vol 239, No. 5, September 1, 2011	All cows diagnosed pregnant at 31 d by ultrasound. 476 randomly allocated not palpated. 230 got single membrane slip, 222 got 2 membrane slips at days 34 to 43.	928 dairy cows	Ultrasound on days 45 and 60	Pregnancy diagnosis via 1 or 2 membrane slips performed during palpation in early gestation did not increase pregnancy loss in dairy cattle.