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THE FUTURE OF PREGNANCY TESTING IN BEEF CATTLE

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Introduction

Pregnancy testing has evolved as an important management tool for most beef producers in the western United States, particularly in larger operations. In most beef herds, the purpose for pregnancy testing is for assessment of the overall reproductive program and to look at marketing opportunities. Marketing opportunities may exist for the sale of the non-pregnant or open cow in addition to late-bred females that no longer fit into a given operation. This is driven by the high costs of maintaining a non-productive cow in most management systems. Beef cattle are usually checked for pregnancy from 50-90 days after the end of breeding season but sometimes earlier if justified. Requirements for pregnancy testing needed by the producer are accuracy, efficiency, and affordability. Back a few years, it was reported in a national survey of beef producers they expected at least a 95% accuracy of their veterinarian in pregnancy examinations. This is certainly below the expectations I deal with in practice. Other national survey information indicates less than 20% of herd owners examine cows or heifers for pregnancy (National Animal Health Monitoring System, 1994). This again is far below my current observation of its use in the western United States.

Methods of Pregnancy Evaluation

Rectal Palpation

Palpation *per rectum* (rectal palpation) has been in widespread usage for pregnancy evaluation since the early 1900s (Roberts, 1986). With a skilled examiner, pregnancy can be determined as early as 30-35 days of gestation (Ball, 1980). Four positive signs of pregnancy are used in the field. The four positive signs are:

- Palpation of the amniotic vesicle
- Palpation of the fetus
- Palpation of placentomes
- Palpation of membrane slip

Amniotic vesicle. The amniotic vesicle is comprised of the amnion membrane plus fluid surrounding the developing conceptus. It is palpable as early as 28-32 days after conception in heifers and 32-35 days in older cows. It is recognized early as a spherical, turgid, fluid-filled structure. After approximately 50 days after conception, the vesicle elongates and is more oval in structure. Between 62-65 days the amnion is softening and the borders become less distinct. The vesicle can be used to estimate duration of pregnancy fairly accurately between 35 and 65 days of pregnancy.

Table 1. Estimation of pregnancy duration by measurement of the Amniotic vesicle

Duration in days	Metric (cm)	Width (Finger or hand)*
35	0.7	½ finger tip
42	1.5	1 finger tip
48	3.5	2 finger tips
53	5.5	3 finger tips
58	7.5	4 finger tips
62	9.0	Palm less thumb
65	10.5	Hand and thumb

*Average hand

Adapted from L. Ball. Pregnancy Diagnosis in the Cow. Current Therapy in Theriogenology. 1980

Fetus. The fetus becomes palpable around days 65 to 70 of pregnancy as the amniotic vesicle softens and allows this manipulation. From this point, the fetus becomes a valuable tool in positive diagnosis and timing of duration of pregnancy. Measurement of the crown-nose of the fetal head is especially valuable in this regard.

Table 2. Estimation of pregnancy duration by fetal crown to nose measurements

Duration in days	**Metric (cm)**	**Width (Finger or hand)***
68-70	1.5	1 finger tip
80	3.5	2 finger tips
90	5.5	3 finger tips
100	7.5	4 finger tips
110	9.0	Palm less thumb
120	10.5	Hand and thumb

*Average hand

Adapted from L. Ball. Pregnancy Diagnosis in the Cow. Current Therapy in Theriogenology. 1980

The fetus can generally be palpated within the uterus for the first 4 months of gestation. After this, the fetus will generally be deep in the uterus and out of reach until approximately 7 months of gestation when it may be balloted within the uterus. From about 6 ½ months of gestation the fetus may be balloted from outside the cow from the right side by pushing against the uterine wall and putting the uterine fluids in motion. Often the fetus will rebound against you hand and can be felt as a hard object that bounces of your hand.

Placentomes. Placentomes arise from fusion of the cotyledons of the fetal placenta with the maternal caruncle. They begin to form early in gestation and are usually palpable by day 75-80 of pregnancy. The size of the placentomes varies with duration of pregnancy and uterine location. They are more inconsistent than either fetus or amniotic vesicle in estimating duration of pregnancy. If using for duration of pregnancy, it is recommended measurements be made of placentomes just in front of the cervix. The tendency is to generally underestimate the size of the placentomes. Care also must be used to make sure you are feeling placentomes and not ovaries. General guidelines

suggest the placentomes about "dime" size at 90 days, "nickel" at 105 days, "quarter" at 120, and "fifty-cent" piece size at 150 days but a lot of variation can occur.

Fetal membrane slip. The examiner can detect the developing placenta (fetal membrane) within the lumen of the pregnant uterus as early as 30 days. This is done by compressing the pregnant horn and allowing the fetal membranes and uterine wall to "slip" between the thumb and forefinger. This will generally feel similar to the slipping of a string or fold within the lumen of the uterus as the wall of the uterus is compressed together. This has to be done gently as it can causes severe losses of pregnancy in early gestation. **(Opinion- By the time you can really use this safely as a palpation method you have other positive signs that should be used)** Fetal membrane slip cannot be used in any way to estimate duration of pregnancy.

Effect on fetus. One should recognize it is difficult to separate fetal attrition from rectal palpation from spontaneous fetal death. Palpation of the pregnant uterus should be as gentle as possible. Palpation of the early pregnant cow or heifer increases the risk for fetal damage or losses of pregnancy particularly if it involves rigorous palpation of the amnion and/or fetal membrane slip (Abbitt *et al.*, 1978, Vaillancourt *et al.*, 1979; Paisley *et al.* 1987; Ducharme *et al.*, 1990). Differences exist between examiners both in physical attributes, attitudes, and abilities. Tables 3-5 present some information on fetal attrition associated with rectal palpation.

Table 3. Effect of method of rectal palpation on loss of pregnancy

	TRIAL 1		TRIAL 2	
Method of Palpation	No.	% Loss	No.	% Loss
Fluctuation ONLY	138	5.8	289	3.8[a]
Fluctuation and Amnion	139	6.5	333	6.0[ab]
Fluctuation and Membrane Slip	144	9.0	307	9.1[b]

[ab]Within column, values with different superscripts differed P<0.025
Abbitt *et al*, JAVMA, 1978

Table 4. Interaction of days of palpation and method of palpation on loss of pregnancy

	35-51 days		52-70 days	
Method of Palpation	No.	% Loss	No.	% Loss
Fluctuation ONLY	140	4.3	136	3.7
Fluctuation and Amnion	206	7.8	117	1.7
Fluctuation and Membrane Slip	136	14.0	157	5.1
Total	482	8.5[a]	410	3.7[b]

[ab]Within rows, values with different superscripts differed P<.05
Abbitt *et al*, JAVMA, 1978

Table 5. Interaction of Clinician and Method of Palpation on loss of pregnancy

	Clinician					
	A		B		C	
Method of Palpation	No.	% Loss	No.	% Loss	No.	%Loss
Fluctuation ONLY	122	1.6	75	5.3	79	6.3
Fluctuation and Amnion	106	3.8	91	5.5	126	8.7
Fluctuation and Membrane Slip	107	7.5	99	8.1	87	12.6
Total	335	4.2[a]	265[ab]	6.4	292	9.2[b]

[ab]Within rows, values with different superscripts differed at P<0.025
Abbitt *et al*, JAVMA, 1978

Ultrasound

The use of real-time ultrasonography as a method of pregnancy diagnosis has been extensively described and is in widespread use within the dairy industry and to a lessor degree within the beef cattle industry (Beal *et al.*, 1992, Fricke, 2002, Lamb and Fricke, 2005). Ultrasound may be accurate as early as day 27 of pregnancy and requires a skilled technician. Currently, in our teaching program we teach ultrasound as well as rectal palpation to our students as a primary method of pregnancy evaluation. We have found this to be an excellent teaching tool even to teach pregnancy diagnosis by rectal palpation. Information on embryo/fetal losses is limited. The advantage of ultrasound in the detection of early pregnancy in the beef cattle industry is somewhat negated by early embryonic losses.

Table 6. Reported Embryonic/Fetal Mortality in cows or heifers after initial diagnosis of pregnancy by ultrasound followed by reexamination at a later date.

No. Pregnant/ Days of Gestation	Reexamination No. Pregnant/ Days of Gestation	Embryonic/Fetal Mortality
Beal et al., 1992 *(Cows)*		
138	129	6.5
25 days	45 days	
129	127	1.5
45 days	65 days	
138	127	8.0
25 days	65 days	
Lamb el al., 1997 *(Heifers)*		
149	143	4.0
30 days	60 days	
271	260	4.1
35 days	75 days	
105	100	4.8
30 days	90 days	

Chemical Methods

Chemical methods have been developed to detect the very early non-pregnant cow to facilitate rebreeding. Their application in most beef operations would have limited merit. They are presented here as alternative methods of pregnancy evaluation.

Progesterone. Detection of progesterone has also been used to determine pregnancy status in cows (Booth et al., 1979, Dobson and Fitzpatrick, 1976, Macfarlane et al., 1977). Because most estrous cycles are 16 to 24 days in length with an average of 21 days, it is possible to sample most non-pregnant cows at a time progesterone concentration is low, which makes them distinguishable from pregnant cows that have elevated progesterone concentration. This has been most accurate in predicting pregnancy if applied to cows 21-24 days post AI.

Early pregnancy factor. Other peripheral indicators of early pregnancy in the cow include blood levels of early pregnancy factor (EPF) (Cordoba et al., 2001, Grandy et al., 2001) and pregnancy-specific protein B (PSPB) (Austin et al., 1999). EPF is made of EPF-A secreted from the oviduct and EPF-B from the ovary. A signal from the fertilized egg is needed for production of EPF-B. Use of EPF, also referred to as early conception factor (ECF), as a diagnostic for early pregnancy has been problematic (Cordoba et al., 2001, Grandy et al., 2001).

Pregnancy associated glycoprotein. Recognition of pregnancy by the dam has provided and opportunity to look at another group of chemicals to detect pregnancy. Detection of these chemical(s) could be a useful method of pregnancy determination because of specificity of the chemical.

The embryo proper and trophoblast (conceptus) releases signals to maintain production of progesterone by the corpus luteum during early pregnancy (Spencer and Bazer, 2002, Thatcher et al., 2005). During the period of maternal recognition of pregnancy in cows (days 15-18), the conceptus is free-floating in the uterus. Interferon (IFN)-τ is a major signal produced by the bovine conceptus and acts on the endometrium to elicit secondary responses necessary to maintain pregnancy (Roberts et al., 1992, Spencer and Bazer, 2002, Thatcher et al., 1995). Conceptus-derived IFN-τ disrupts the signal transduction pathway that regulates release of the major luteolytic product in cattle, prostaglandin F2α (Thatcher et al., 1995, Thatcher et al., 2001). Interferon-τ also induces synthesis and secretion of a protein called ISG15 (named based on induction of a 17-kDa protein as described by HUGO Gene Nomenclature). ISG15 is induced in the uterus on days 15-45 pregnancy in cows(Austin et al., 1996, Hansen et al., 1997, Johnson et al., 1998, Perry et al., 1999, Thatcher et al., 2001). While it has been shown to be induced by IFN-τ in ruminants, the cytokine or growth factor that induces ISG15 in mice and primates has not been described. In a study, to determine the efficacy of using ISG15 as a marker (Han et al., 2006*),* serial blood were collected either on day 18 (n=78) or days 15-21, 25, and 32 from dairy cows following artificial insemination (AI). Samples were analyzed for both progesterone and ISG15. Transrectal ultrasound for pregnancy at day 32 was used as the control. ISG15 mRNA levels increased after day 16, peaked at day 20 and then declined to day 16 levels by 32 days following AI. The average pregnancy rate was 43% based on blood cell ISG15 mRNA. The average pregnancy rate was 33% based on transrectal ultrasound. Detection of ISG15 mRNA yielded 78% accuracy in predicting pregnant cows, while progesterone yielded 58% accuracy. This test is a more accurate indicator of cows not pregnant. Lower levels of ISG15 mRNA during serial collections

were 100% accurate in predicting non-pregnant cows based on day 32 transrectal ultrasound.

Pregnancy associated glycoprotein-1 (PSPB) al (Roberts et al., 1995) is accurate in predicting pregnancy after day 30 (Szenci et al., 1998). Pregnancy-Specific Protein B (PSPB) has an exceptionally long half-life and remains in circulation for several months following parturition, affecting the PSPB as a marker for pregnancy when cows are inseminated prior to 70 days post partum (Austin et al., 1999, Kiracofe et al., 1993). Pregnancy diagnosis using PSPB is available commercially (BioPRYN^{TM}; BioTracking, LLC). Samples are taken 28 days and 30 days post insemination in heifers and cows, respectively. Samples are sent to a laboratory for assay.

The practical application of these methods in the beef industry is yet to be determined.

Conclusion

Industry needs will dictate the application of the various methods of pregnancy determination. In our practice we have found both rectal palpation and ultrasonography to be both fast and accurate. Approximately 50% of 6,200 heifers and 15% of the 18,000-20,000 mature beef cows are examined by ultrasound. Most is simply to determine pregnancy status. A small amount of requests are for duration of pregnancies and sexing of fetuses for sale purposes. Because of the time involved in sample collection assaying and information return, limited usage is made of chemical methods at this point. This will continue to be the case in the beef industry unless we get more accuracies, efficiencies and lower costs.

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