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ULTRASOUND – EARLY PREGNANCY DIAGNOSIS AND FETAL SEXING

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Introduction

The area that has arguably benefited more from the development of ultrasound technology than any other area is reproduction in large animals. In many cases, rectal palpation has been replaced by transrectal ultrasonography for pregnancy determination, and diagnoses associated with uterine and ovarian infections. In addition, ultrasonography has added benefits such as fetal sexing, early embryonic detection and is less invasive than rectal palpation. From a research standpoint, ultrasound has given us the ability to visually characterize the uterus, fetus, ovary, corpus luteum, and follicles. More accurate measurements of the reproductive organs has opened doors to new areas of research and validated or refuted data from past reports.

Practical applications of ultrasound by bovine practitioners for routine reproductive examinations of cattle is the next contribution this technology is positioned to make to the livestock industry. Most veterinary students continue to be taught that ultrasound is a secondary technology for bovine reproductive work; however, the information-gathering capabilities of ultrasonic imaging far exceed those of rectal palpation (Ginther, 1995). This paper will discuss the impact and practical applications of ultrasound for conducting routine reproductive examinations in dairy cattle.

Imaging the Bovine Uterus and Conceptus

Of all the ultrasound applications utilized by technicians in the industry, scanning of the uterus for infection and pregnancy are the most commonly practiced commercial applications that we have seen in the cattle industry. In a nonpregnant, cycling cow the uterine tissue appears as a somewhat echogenic structure on the screen. Because the uterus is comprised of soft tissue it absorbs a portion of the ultrasound waves and reflects a portion of the waves. In this way we can identify the uterus as a gray structure on the screen. A cross-sectional view of the uterus is displayed as a "rosette" and is easily distinguished from other peripheral tissues, whereas the longitudinal section is less recognizable, yet a trained technician can differentiate between the elongated view of the uterus and other tissues that may appear similar. Physiological changes during the estrous cycle leads to physical changes (such as tone) in the uterus, which alters the echogenic properties of the uterus (Pierson and Ginther, 1987a). Even though a scoring system has been developed to describe changes in uterine echogenic ability during different stages of the estrous cycle (Pierson and Ginther, 1987a), predicting the stage of the estrous cycle remains inconsistent.

Pathological applications for ultrasound technology have extended to identifying endometritis, pyometra, mucometra, and hydrometra (Perry et al., 1990). With the aid of ultrasound, researchers have determined that uterine infections were related to delayed postpartum folliculogenesis (Peter and Bosu, 1988), to the occurrence of short luteal phases after the first postpartum ovulation (Peter and Bosu, 1987), and to the development of follicular cysts on the ovaries (Peter and Bosu, 1987).

Detection of the embryo proper as well embryonic and fetal developmental characteristics during early fetal development are shown in Table 1 and 2. The bovine fetus can be visualized beginning at 20 d post breeding and continuing throughout gestation, however, because of its size in relation to the image field of view, the fetus cannot be imaged *in toto* after about 90 days using a 5.0 MHz linear-array transducer.

Table 1. Day of first detection of ultrasonographically identifiable characteristics of the bovine conceptus (Adapted from Curran et al., 1986).

	First day detected	
Characteristic	**Mean**	**Range**
Embryo proper	20.3	19 to 24
Heartbeat	20.9	19 to 24
Allantois	23.2	22 to 25
Spinal cord	29.1	26 to 33
Forelimb buds	29.1	28 to 31
Anmion	29.5	28 to 33
Eye orbit	30.2	29 to 33
Hindlimb buds	31.2	30 to 33
Placentomes	35.2	33 to 38
Split hooves	44.6	42 to 49
Fetal movement	44.8	42 to 50
Ribs	52.8	51 to 55

Early Pregnancy Diagnosis

Reports have indicated the detection of an embryonic vesicle in cattle as early as 9 (Boyd et al., 1988), 10 (Curran et al., 1986a), or 12 days (Pierson and Ginther, 1984) of gestation. In these situations the exact date of insemination was known and ultrasonography simply was used as a confirmation of pregnancy or to validate that detection of an embryo was possible within the first two weeks of pregnancy. In contrast, Kastelic et al. (1989) monitored pregnancy in pregnant and nonpregnant yearling heifers that were all inseminated. Diagnosis of pregnancy in heifers on day 10 through day 16 of gestation resulted in a positive diagnosis for pregnant or nonpregnant of less than 50%. On days 18, 20, and 22 of gestation accuracy of pregnancy diagnosis improved to 85%, 100%, and 100%, respectively. Although evidence of a pregnancy via ultrasound during days 18 to 22 of gestation yields excellent results, a technician needs to ensure that confusion between fluid accumulation in the chorioallantois during early pregnancy (Kastelic et al., 1989) and uterine fluid within the uterus during proestrus and estrus are not confused when making the diagnosis.

Several further reports (Taverne et al., 1985; Hanzen and Delsaux, 1987; Pieterse et al., 1990, Badtram et al., 1991) also indicate the presence of an embryonic vesicle as early as day 25 of gestation. Although Hanzen and Delsaux (1987) utilized a 3.0 MHz transducer for pregnancy diagnosis, they concluded that by day 40 of gestation a positive diagnosis of pregnancy was 100% accurate, whereas overall diagnosis of pregnancy and absence of pregnancy from day 25 of gestation proved to be correct in 94% and 90% of cases, respectively. In 148 dairy cows, pregnancy diagnosis from day 21 to day 25 was 65% accurate, whereas diagnosis of pregnancy from day 26 to day 33 was 93% accurate (Pieterse et al., 1990). In their conclusions, the authors state that probable causes of misdiagnosis from day 21 to day 26 were either an accumulation of proestrus or estrus uterine fluid, or the accumulation of pathological fluid in the uterus, or were diagnosed pregnant but experienced early embryonic loss.

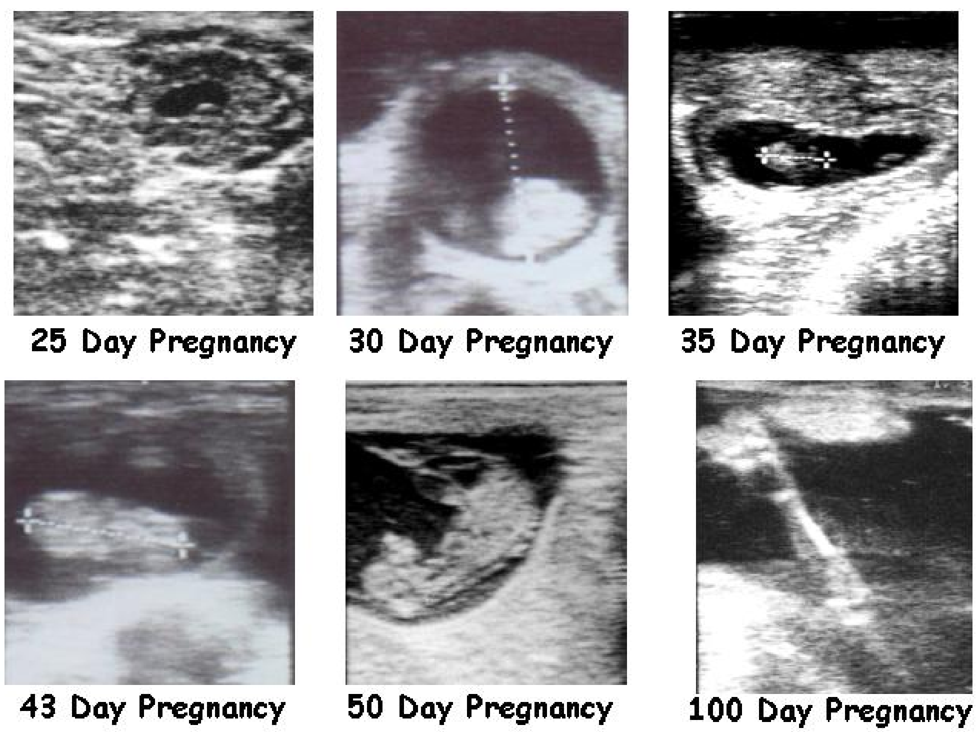

Figure 1. Ultrasound images of the bovine fetus at various stages of development (Lamb, 2001).

Although we have indicated that an embryonic vesicle is detectable by ultrasound as early as 9 days of gestation, accuracy of detection approaches 100% after day 25 of gestation. For practical purposes, the efficiency (i.e., speed and accuracy) of a correct diagnosis of pregnancy should be performed in females expected to have embryos that are at least 26 days of age (Figure 1). This information can be used to determine the age of bovine fetuses with a high degree of accuracy (Pierson and Ginther, 1984; Boyd et al., 1988; Ginther, 1995). Crown-Rump length measurements were summarized by Hughes and Davies (1989; Table 3). There was a significant correlation (r = 0.98) between embryo age and crown-rump length.

Table 2. Fetal crown-rump length in relation to age in weeks (Hughes and Davies, 1989).

		Crown-rump length, mm		
Fetal age, weeks	**No. of observations**	**Minimum**	**Maximum**	**Mean**
4	25	6	11	8.9
5	35	8	19	12.8
6	50	16	26	20.2
7	47	23	36	27.7
8	41	36	52	45.5
9	48	39	71	62.4
10	43	61	101	87.4
11	39	95	118	106.5
12	32	107	137	121.8

Ultrasound is a rapid method for pregnancy diagnosis, and experienced palpators adapt to ultrasound quickly. The time required to assess pregnancy in beef heifers at the end of a 108-day breeding season averaged 11.3 seconds using palpation per rectum versus 16.1 seconds required to assess pregnancy and fetal age using ultrasound (Galland et al., 1994). Fetal age also affected time required for diagnosis with older fetuses requiring less total time for diagnosis (Galland et al., 1994). Although ultrasound at ≥45 d of gestation did not increase accuracy of pregnancy diagnosis for an experienced palpator, it may improve diagnostic accuracy of a less experienced one (Galland et al., 1994).

Two caveats must be considered when using ultrasound for routine early pregnancy diagnosis in a cow herd. First, when using ultrasound for early pregnancy diagnosis, emphasis must be given to identifying nonpregnant rather than pregnant cows. Second, a management strategy must be implemented to return the nonpregnant cows to service as quickly as possible after pregnancy diagnosis. Such strategies include administration of $PGF_{2\alpha}$ to cows with a responsive CL, use of estrus detection aids, or a combination of both methods.

Early Embryonic Loss

Prior to the development of ultrasound for pregnancy diagnosis in cattle, technicians were unable to accurately determine the viability or number of embryos or fetuses. Because the heartbeat of a fetus can be detected at approximately 22 days of age, we can accurately assess whether or not the pregnancy is viable. Studies in beef (Diskin, M.G. and J.M. Sreenan. 1980; Beal et al., 1992; Lamb et al., 1997) and dairy (Smith and Stevenson, 1995; Vasconcelos et al., 1997; Fricke et al., 1998; Szenci et al., 1998) cattle have used ultrasound to assess the incidence of embryonic loss. The number of fetuses can most accurately be assessed at between 49 and 55 days of gestation (Davis and Haibel, 1993).

Table 3 summarizes the incidence of embryonic loss by study in beef and dairy females. The fertilization rate after artificial insemination in beef cows is 90%, whereas embryonic survival rate is 93% by day 8 and only 56% by day 12 post artificial insemination (Diskin and Sreenan, 1980). The incidence of embryonic loss in beef cattle appears to be significantly less than in dairy cattle. Beal et al. (1992) reports a 6.5% incidence of embryonic loss in beef cows from day 25 of gestation to day 45. Similarly, Lamb et al. (1997) noted a 4.2% incidence of embryonic loss in beef heifers initially ultrasounded at day 30 of gestation and subsequently palpated rectally at between day 60 and 90 after insemination. In dairy cattle, pregnancy loss from 28 to 56 days after artificial insemination was 13.5%, or 0.5% per day (Fricke et al., 1998). This rate of pregnancy loss is similar to the 12.4% reported by Smith and Stevenson (1995) and the 19.1% reported by Vasconcelos et al. (1997) during a comparable stage of pregnancy in lactating dairy cows. The greatest occurrence of pregnancy loss was between day 28 and 42 of gestation (10.5%) and between day 42 and 56 of gestation (6.3%). After day 56 of pregnancy, embryonic losses were reduced to 3.4% from 56 to 98 days of pregnancy and 5.5% from 98 days to calving (Vasconcelos et al., 1997). Specific physiologic mechanisms responsible for pregnancy loss in dairy cattle may include lactational stress associated with increased milk production (Nebel and McGilliard, 1993), negative energy balance (Butler and Smith, 1989), toxic effects of urea and nitrogen (Butler et al. 1995) or reduced ability to respond to increased environmental temperature (Stevenson et al., 1984; Hansen et al., 1992). These studies indicate the usefulness of ultrasonography as a tool to monitor the success of a breeding program, by determining pregnancy rates and embryonic death.

Additional investigators have reported a range of embryonic mortality from day 21 to 60 to be 8% (Boyd et al., 1969) to 35% (Beghelli et al., 1986). In those reports, embryo mortality was determined by the presence of high blood concentrations of progesterone at day 21 to 23 after breeding (presuming a high concentration of progesterone was caused by the embryonic signal to prevent luteal regression) but the absence of an embryo or fetus by rectal palpation at 40 to 60 days after insemination. The authors rationale assumed that the embryo was lost between day 21 of gestation and the time of palpation; however, there was no positive identification of a viable embryo at day 21 to 23 of gestation. Therefore, ultrasonography provides a tool to accurately differentiate between the failure of a female to conceive or the incidence of embryonic mortality because a heartbeat is detectable at 22 days of gestation.

At present, there is no practical way to reduce early embryonic loss in lactating dairy cows. However, recognizing the occurrence and magnitude of early embryonic loss may actually present management opportunities by taking advantage of new reproductive technologies that increase AI service rate in a dairy herd. If used routinely, transrectal ultrasonography has the potential to improve reproductive efficiency within a herd by reducing the period from AI to pregnancy diagnosis to 26 to 28 days with a high degree of diagnostic accuracy.

Table 3. Incidence of embryonic/fetal loss in cows after an initial diagnosis of pregnancy by ultrasound, followed by a second diagnosis prior to or at calving

Reference	No. pregnant, days of gestation	No. pregnant, days of gestation	No. of embryos lost	Embryonic mortality, %
Beef Cattle				
Beal et al., 1992 *(Cows)*	138 25 days	129 45 days	9	6.5
	129 45 days	127 65 days	2	1.5
	138 25 days	127 65 days	11	8.0
Lamb et al., 1997 *(Heifers)*	149 30 days	143 60 days	6	4.0
	271 35 days	260 75 days	11	4.1
	105 30 days	100 90 days	5	4.8
Dairy Cattle				
Smith and Stevenson, 1995 *(Cows and Heifers)*	129 28 to 30 days	113 40 to 54 days	16	12.4
Vasconcelos et al., 1997 *(Cows)*	488 28 days	437 42 days	51	10.5
	437 42 days	409 56 days	28	6.3
	409 56 days	402 70 days	7	1.7
	402 56 days	395 70 days	7	1.7
	488 28 days	395 98 days	93	19.1
Fricke et al., 1998 *(Cows)*	89 28 days	77 56 days	12	13.5
Szenci et al., 1998 *(Cows)*	64 26 to 58 days	52 Full term	12	8.6

Fetal Sexing

Many cattle operations are developing strategies to use fetal sexing as either a marketing or purchasing tool. At approximately day 50 of gestation, male and female fetuses can be differentiated by the relative location of the genital tubercle and development of the genital swellings into the scrotum in male fetuses (Jost, 1971). Fetuses at 48 to 119 days of age have been successfully sexed (Müller and Wittkowski, 1986; Curran et al., 1989; Wideman et al., 1989; Beal et al., 1992). The procedure is reliable and accuracy has ranged from 92 to 100% (Müller and Wittkowski, 1986; Wideman et al., 1989; Beal et al., 1992). Beal et al. (1992) noted that of 85 fetuses predicted to be male 84 were confirmed correct, resulting in 99% accuracy. In addition, of 101 fetuses predicted to be female 98 were confirmed correct, resulting in 97% accuracy. Recently, we (Lamb, 2001) determined the sex of 112 fetuses in Angus heifers with 100% accuracy.

For optimal results the ultrasound transducer should be manipulated to produce a frontal, cross-sectional, or sagittal image of the ventral body surface of the fetus. In larger framed cows (i.e. Holsteins and Continental beef breeds) or older cows the optimum window for fetal sexing usually is between day 55 and 70 of gestation, whereas for smaller framed cows (Jerseys and English beef breeds) the ideal window usually is between day 55 and 80 of gestation. There are two limitations that could inhibit the ability of a technician to determine the sex of a fetus: 1) as the fetus increases in size it becomes more difficult to move the transducer relative to the fetus to obtain the desired image; and, 2) the gravid horn is more likely to descend ventrally into the abdominal cavity in larger or older cows, making fetal sexing virtually impossible without retracting the gravid horn.

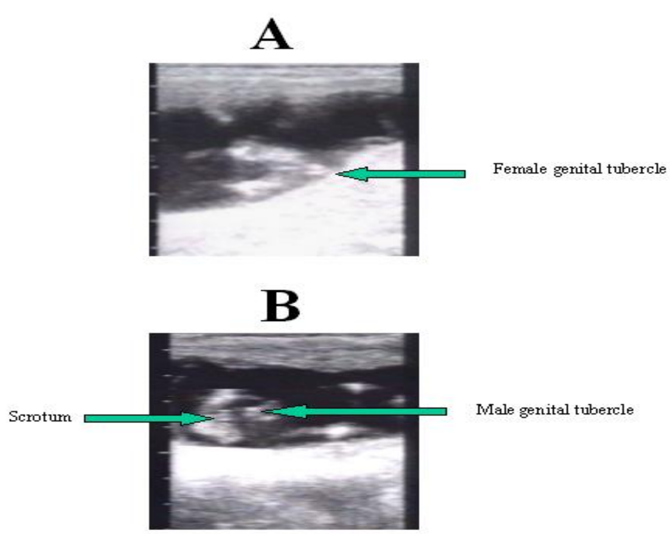

Figure 2. Ultrasound image of a female bovine fetus (65 days of gestation; Panel A) and a saggital view of a male fetus (65 days of gestation; Panel B). Images were taken using a 5.0 Mhz transducer.

Figure 2 illustrates the cross-sectional image of female fetus (65 days of gestation; Panel A) and a saggital view of a male fetus (65 days of gestation; Panel B; Lamb, 2001). The umbilicus can be used as an excellent landmark when determining the location of the genital tubercle or presence of a scrotum in males. In the male, the genital tubercle is located adjacent to and caudal to the umbilicus, whereas the genital tubercle in the female is located just ventral to the tail. The scrotum is detectable between the hind legs of the male fetus. The genital tubercle and scrotum are echogenic and are easily detected on an ultrasound screen as echogenic images. To ensure an accurate diagnosis of sex, for each patient, a technician should view an image at three locations: 1) adjacent to the umbilicus, where the umbilicus enters the abdomen (possible male genital tubercle); 2) the area between the back legs (possible scrotum); and, 3) ventral to the tail (possible female genital tubercle).

In beef cattle operations, fetal sexing remains limited to purebred operations especially in conjunction with an embryo transfer program. Determination of sex especially after the successful transfer of embryos to recipients allows marketing of male and female embryos before the pregnancy is carried to term. This strategy can be used effectively in dairy operations trying to produce bull calves of a particular mating for sale to bull studs. From a commercial cattle operation standpoint, heifer development operations are utilizing fetal sexing as a marketing tool to provide potential buyers with females that are pregnant with fetuses of a specific sex. As more technicians become proficient at fetal sexing, commercial operations will utilize this technology to enhance the marketability and efficiency of their cattle operations.

Conclusion

The full advantages of using ultrasound in cattle operation for pregnancy diagnosis and fetal sexing remain to be discovered, but technology to enhance management practices in progressive operations is available. With repetitive training and a certain degree of skill, technicians are now able to offer services that will enhance profitability of many cattle operations.

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