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PRACTICAL APPLICATIONS OF ULTRASOUND FOR REPRODUCTIVE MANAGEMENT OF BEEF AND DAIRY CATTLE

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Introduction

O. J. Ginther stated: “gray-scale diagnostic ultrasonography is the most profound technological advance in the field of large animal research and clinical reproduction since the introduction of transrectal palpation and radioimmunoassay of circulating hormones.” (Ginther, 1986). It is hard to imagine that many discoveries and procedures related to ovarian, uterine and fetal function that we use today would have been considered without the development of real-time ultrasound. The research and commercial applications of ultrasound developed for reproduction over the last 15 years would support the statement by O. J. Ginther.

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 In many cases, rectal palpation has been replaced by transrectal ultrasonography for pregnancy determination, and diagnoses associated with uterine and ovarian infections.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 beef and dairy 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 and beef cattle.

Veterinary Ultrasound Equipment

In general, linear-array, real-time, B-mode ultrasound scanners are best suited for veterinary applications involving cattle reproduction. Most ultrasound machines consist of a console unit that contains the electronics, controls, and a screen upon which the ultrasound image is visualized by the operator, and a transducer, which emits and receives high-frequency ultrasound waves. Linear-array transducers consist of a series of piezo electric crystals arranged in a row. These crystals emit high frequency sound waves upon being energized. The configuration of a linear-array transducer results in a rectangular image on the field of scan (as opposed to a pie shaped image produced by a sector transducer).

Bovine reproductive organs are most commonly scanned per rectum using a linear-array transducer specifically manufactured for transrectal use. However, specialized applications including ovum pickup and follicle ablation involve a transvaginal approach using a sector transducer. Linear-array transducers of 5.0 and 7.5 MHz frequency ranges are most commonly used in cattle, and most veterinary ultrasound scanners are compatible with probes of different frequencies. Depth of tissue penetration of sound waves and image resolution is dependent upon and inversely related to the frequency of the transducer. Thus, a 5.0 MHz transducer results in greater tissue penetration and lesser image detail, whereas a 7.5 MHz transducer results in lesser tissue penetration and greater image detail. An ultrasound scanner equipped with a 5.0 MHz transducer is most useful for bovine practitioners conducting routine reproductive examinations, however, small ovarian structures such as developing follicles are best imaged with a 7.5 MHz transducer.

It is clear that ultrasound has made a tremendous impact as a scientific tool; however, ultrasound holds much promise as a tool to improve reproductive management in beef and dairy operations. There are several reasons that transrectal ultrasound is not widely used among bovine practitioners at present. First, research-grade ultrasound machines are relatively expensive, costing from $10,000 to $20,000. Second, most ultrasound machines require a cart and an external power source, thereby making them cumbersome to use under field conditions. Recently, several ultrasound manufacturers have developed and marketed ultrasound machines that are cheaper, smaller, and battery operated. At present, these portable ultrasound machines lack the image quality of the larger console based units but may be easier to use on a routine basis. Continuation of the trend toward portability will foster future use of this technology by bovine practitioners for routine reproductive management.

Imaging the Bovine Ovary

Ovarian Structures as Diagnostic Aids

The use of ultrasound technology to evaluate ovarian activity has been reviewed in great detail (Pierson and Ginther, 1988; Beal et al., 1992). Ovarian stroma, ovarian vessels, follicles, cysts, corpora haemorrhagica (CH), and corpora lutea (CL) are all structures that have been previously identified by real-time ultrasonography (Pierson and Ginther, 1988; Kastelic et al., 1990a,b; Beal et al., 1992; Singh et al., 1997). The most distinguishable ovarian structures are antral follicles. Because follicles are fluid-filled structures they absorb ultrasound waves and are displayed as black on the screen (i.e., anechoic or non-echogenic). In contrast, the ovarian stroma, CH, and CL all contain varying degrees of dense cells, which reflect the ultrasound waves and result in a gray image on the screen.

Routine reproductive examinations should include visualization of the major structures (or the lack thereof) on both ovaries. Although rectal palpation can be an accurate method for diagnosing pregnancy, rectal palpation is a poor method for resolving ovarian follicles (Pieterse et al., 1990). By contrast, ultrasonic imaging is a highly accurate and rapid method for assessing ovarian structures (Griffin and Ginther, 1992). Too often, bovine practitioners proceed directly to scanning the uterus during reproductive examinations and neglect the ovaries all together. This is unfortunate because the ovaries contain a wealth of information that can be used to aid in diagnosing the reproductive status of the cow and for selecting appropriate therapies or reproductive interventions. For example, presence or absence of a corpus luteum aids in diagnosing pregnancy status, especially when conducting pregnancy exams early post-AI. When present, the size and location (i.e., left vs. right ovary) of the corpus luteum indicates the location of the conceptus within the uterus if the cow is pregnant. Because most twinning in cattle is dizygous (Wiltbank et al., 2000), the presence of multiple corpora lutea is a diagnostic indicator of the presence of twin fetuses. Ovarian pathologies such as "static ovaries" and follicular and luteinized cysts can easily be distinguished. Use of ovarian structures as diagnostic aids during reproductive examinations, however, requires a thorough understanding of ovarian and reproductive anatomy and physiology. In addition, there are limitations to the conclusions that can be made from a single (as opposed to serial) ultrasound examination.

Ovarian Follicles

Folliculogenesis is the process of forming mature follicles capable of ovulation from the pool of nongrowing, primordial follicles in the ovary (Spicer and Echternkamp, 1986). Ovarian follicles are fluid-filled structures surrounded by an inner layer of granulosa cells and an outer layer of thecal cells. The oocyte is suspended within the antrum by a specialized pedicle of granulosa cells called the cumulus oophorous. Because fluid absorbs rather than reflects ultrasound waves, fluid-filled structures such as follicles appear as black circular structures surrounded by echogenic ovarian tissue. Most veterinary grade ultrasound scanners can resolve ovarian follicles with a diameter of 2 to 3 mm or greater, and larger follicles can easily be tracked during serial scanning sessions (Pierson and Ginther, 1988). The ability to nonivasively track follicular growth during the estrous cycle using ultrasound has revolutionized our understanding of reproductive physiology.

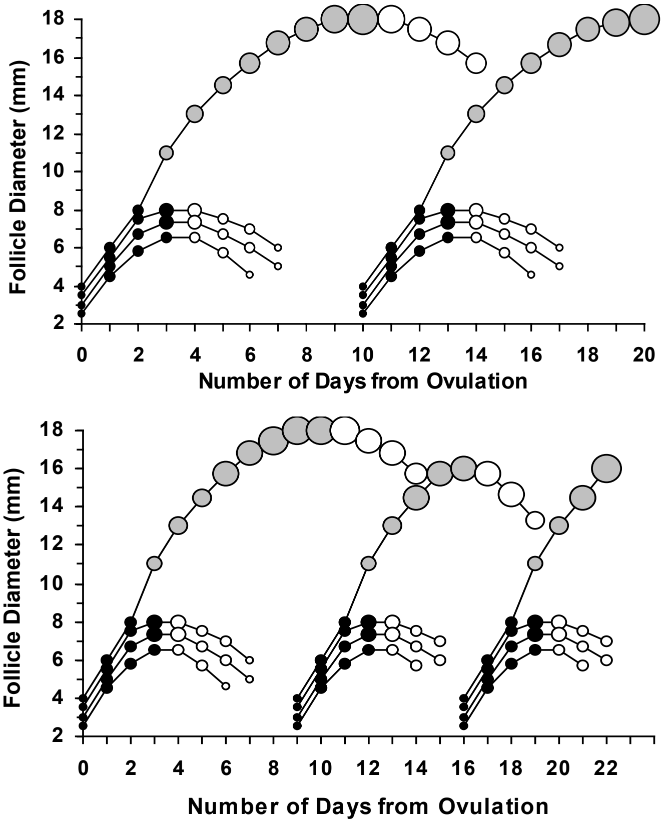

Figure 1. Schematic diagram depicting two-wave (top panel) and three-wave (bottom panel) patterns of follicular growth during the bovine estrous cycle. Growing follicles before selection of the dominant follicle are depicted as black circles, the dominant follicles of each wave are depicted as gray circles, and atretic follicles are depicted as open circles.

Follicular Waves

Scientific studies using transrectal ultrasonography have led to clarification of the nature of antral follicular development in cattle (For a review, see Ginther et al., 1996). The first studies using ultrasound revealed that follicular growth occurs in waves, each wave culminating with formation of a large follicle (Figure 1).

A follicular wave begins with emergence of a group or cohort of small antral follicles just before the day of ovulation. During the next several days, one of the follicles in this cohort continues to grow and becomes dominant, thereby suppressing subordinate follicles within the wave from which it originated as well as emergence of follicles in an ensuing follicular wave. As the dominant follicle continues to grow, growth of the remaining follicles in the cohort ceases or slows, and these subordinate follicles eventually undergo atresia. A second wave of growth emerges on approximately Day 10 after ovulation and, for three-wave cycles, an additional wave emerges at Day 16 after ovulation. For both two and three-wave cycles, the ovulatory follicle arises from the final wave (Ginther et al., 1996).

Manual palpation or ultrasonographic examination of the cow's genital tract are currently used by veterinarians involved in reproductive management. A recent report (Aslan et al., 2000) evaluating the difference in detection of follicles by ultrasound or rectal palpation concluded that ultrasound was more effective at identifying follicles greater than 10 mm in diameter than rectal palpation. Follicles 10 to 15 mm in diameter were detected in 90% of cases using ultrasonography versus 62% of the cases using rectal palpation. Follicles greater than 15 mm were detected in 100% of the cases for both ultrasonography and rectal palpation. In a similar review (Hanzen et al., 2000) manual diagnosis of follicles <10 mm was inaccurate, but ultrasound offered the possibility to diagnose follicles <5 mm and to measure the diameter of those follicles. Figure 2 demonstrates the appearance of the ovary at various stages of follicular development prior to emergence of a follicular wave, during proestrus, and after development of a follicular cyst.

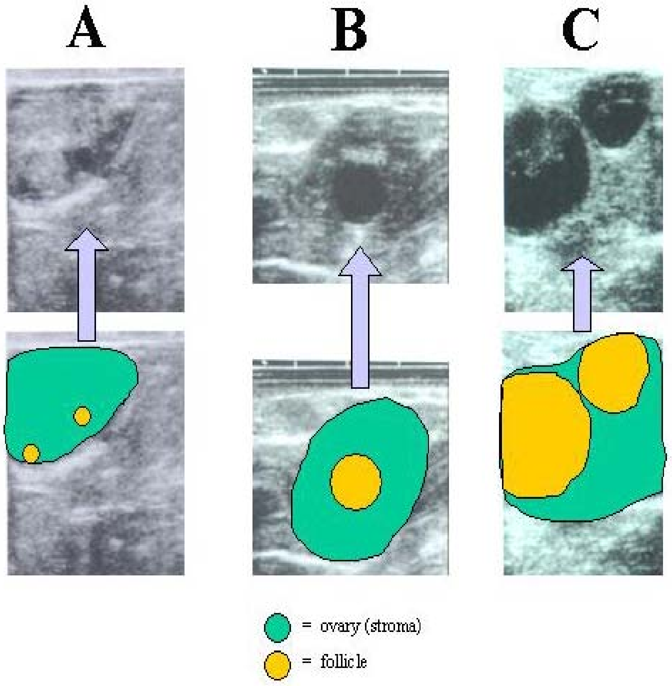

Figure 2. Ultrasound image of bovine ovaries prior to emergence of a follicular wave (note two small follicles [< 5 mm]; Panel A), during proestrus (note pre-ovulatory follicle [13 mm]; Panel B), and after development of a follicular cyst (note delamination of granulose layer into the antrum; Panel C). Images were taken using a 7.5 Mhz transducer (Lamb, 2001).

Corpora Lutea

The CL is a transient endocrine gland that forms after ovulation from the tissues that previously composed the ovarian follicle. Thus, the CL can be viewed as the terminal stage of follicular development. Corpora lutea appear as distinctly echogenic areas within the ovarian stroma. Many corpora lutea appear as a solid tissue masses but may also contain fluid-filled cavities. Based on ultrasonographic examinations in dairy heifers, 79% of otherwise normal CL contain cavities ranging from less than 2 to greater than 10 mm in diameter at some time during the estrous cycle and early pregnancy (Kastelic et al., 1990b; Singh et al., 1997). The appearance of the CL may be used to estimate the stage of the bovine estrous cycle (Kastelic and Ginther, 1989; Kastelic et al., 1990a,b; Singh et al 1997), yet differences in CL development decrease the accuracy of estimates. A higher percentage of corpora lutea in early diestrus tend to have a fluid filled lumen versus the corpora lutea during late diestrus and advanced stages of pregnancy. We (Spell et al., 2001) determined that luteal diameter was not associated with concentrations of progesterone on day 7 of the estrous cycle, but area and volume were correlated to concentrations of progesterone.

Ultrasonographic attributes of CL including cross-sectional diameter, luteal area, and echogenicity have been correlated to luteal structure and function (Battocchio et al., 1999; Kastelic et al., 1990a; Singh et al., 1997). Use of luteal characteristics to improve accuracy of pregnancy diagnosis has been reported in dairy heifers (Kastelic et al., 1991), but similar data does not exist for beef or lactating dairy cattle. Luteal size and echogenic characteristics assessed at specific times post breeding may prove useful as a method to improve accuracy of early pregnancy diagnosis in dairy cattle. Although ultrasound is more accurate than rectal palpation for assessing ovarian follicles, it is difficult to distinguish between developing corpora lutea and older regressing corpora lutea using either technique (Pieterse et al., 1990).

Ovarian Cysts

For beef cattle the diagnosis of cysts is of little practical importance, whereas, diagnosis of cysts in dairy cattle most often occurs during routine postpartum rectal examinations conducted by a bovine practitioner. Palpation per rectum of a large, fluid-filled structure is commonly used as a clinical indication of a follicular cyst. Differentiation between follicular and luteal cysts via rectal palpation is difficult, even for experienced practitioners (Dawson, 1975; Farin et al., 1992). Accuracy of diagnosis increases when using transrectal ultrasonography, with correct identification of greater than 90% of luteal and nearly 75% of follicular cysts (Farin et al., 1990, 1992). Follicular and luteal cysts also can be classified based on serum progesterone concentrations (Farin et al., 1990). Diagnosis of a cyst in conjunction with low serum progesterone is indicative of a follicular cyst, whereas a cyst in conjunction with high serum progesterone is indicative of a luteal cyst. Using these criteria, a benign follicular cyst would fall into either category depending on the stage of the estrous cycle when they were detected.

Treatment for ovarian cysts depends on the classification of the cyst. Follicular cysts are most commonly treated by administration of synthetic GnRH analogs approved for use in lactating dairy cows (Bierschwal et al., 1975; Seguin et al., 1976; Whitmore et al., 1979). Manual rupture of cysts via rectal palpation is not recommended because adverse side effects including adhesions around the ovary and adnexa may impair fertility (Archibald and Thatcher, 1992). Interestingly, approximately 20% of untreated cows with follicular cysts recover spontaneously (Bierschwal et al., 1975), supporting the notion that many of these cysts may be benign. Treatment with GnRH induces luteinization rather than ovulation of the follicular cyst, and ultimately results in formation of a luteal cyst (Garverick, 1997). Once formed, regression of a luteal cyst can be induced by administration of $PGF_{2\alpha}$ (Nanda et al., 1988). Administration of GnRH to cows with benign follicular cysts often induces ovulation of a normally growing dominant follicle rather than the cyst itself (Fricke and Wiltbank, 1999, Table 1), and other researchers have reported similar observations (Archibald and Thatcher, 1992; Garverick, 1997).

Table 1. Effect of ovarian cysts on synchronization rate and conception rate in lactating dairy cows after synchronization of ovulation using Ovsynch (Adapted from Fricke and Wiltbank, 1999).

	Ovarian cyst[a]		
Item	**Yes**	**No**	**Overall**
Incidence	11.0%	89.0%	
Synchronization rate[b]	73.1%	85.3%	84.0%
Conception rate[c]	36.8%	48.8%	47.6%

[a] A fluid-filled ovarian cyst ≥ 25 mm in diameter present at the time of the second GnRH injection of the Ovsynch protocol.

[b] Ovulation of a normal dominant follicle after the second GnRH injection of the Ovsynch protocol.

[c] Ultrasonographic determination conducted at 28 d post AI.

Ovsynch, a protocol for synchronizing ovulation in lactating dairy cows, uses injections of GnRH and $PGF_{2\alpha}$ (Pursley et al,. 1995, 1997) and is an effective treatment for ovarian cysts. A recent field trial using Ovsynch and ultrasonographic monitoring of ovarian structures (Table 1) revealed that 11% of lactating cows exhibited a large ovarian structure that would have been diagnosed as a cyst using rectal palpation. Treatment with Ovsynch induced ovulation of a follicle other than the cyst that was present at the time of the second GnRH injection in 73% of cows, and nearly 37% of these synchronized cystic cows conceived after a timed AI. Thus, Ovsynch the treatment of choice for establishing pregnancy in lactating dairy cows exhibiting ovarian cysts. Data from a separate study has recently corroborated these findings (Bartolome et al., 2000).

Diagnostic Limitations of Ultrasonic Imaging

Under most circumstances, practical application of ultrasound for routine reproductive management consists of a single ultrasound examination at a given point in time. It is important to understand that the physiological status of a follicle (e.g., dominant, subordinate, growing, regressing) or corpus luteum cannot be determined during a single ultrasound exam. In addition, ultrasonic imaging aids in distinguishing anatomical attributes of a structure but confers little information regarding physiological or endocrine status. For example, ovarian cysts can be categorized by anatomical attributes such as diameter and presence or absence of luteal tissue; owever, no information regarding functionality such as plasma hormone concentrations can be conferred. One exception would be the visualization of a fetal heartbeat as a diagnostic indicator of a viable fetus. The diagnostic limitation of ultrasonic imaging becomes important especially when the limitation is exceeded and an incorrect therapy or reproductive intervention is recommended. A thorough understanding of ovarian physiology and the mechanisms by which hormonal programs succeed or fail is imperative for correct interpretation of ultrasonic imaging information.

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 (Figure 3). 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.

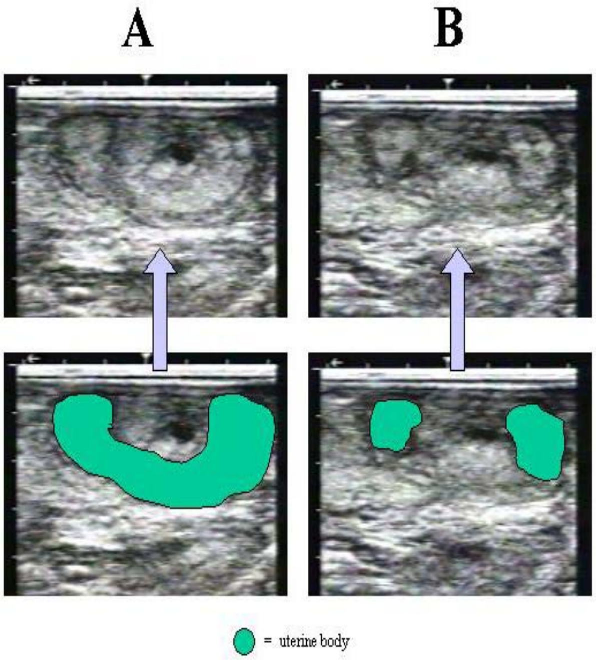

Figure 3. Ultrasound image depicting an elongated (Panel A) and cross-sectional (Panel B) view of the non-pregnant uterus. Images were taken using a 5.0 Mhz transducer (Lamb, 2002=1).

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 2 and 3. 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 2. 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.

25 Day Pregnancy 30 Day Pregnancy 35 Day Pregnancy

43 Day Pregnancy 50 Day Pregnancy 100 Day Pregnancy

Figure 4. Ultrasound images of the bovine fetus at various stages of development.

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

Fetal age, weeks	No. of observations	Crown-rump length, mm		
		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 4 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 cattle. 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. 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 4. 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 5 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.

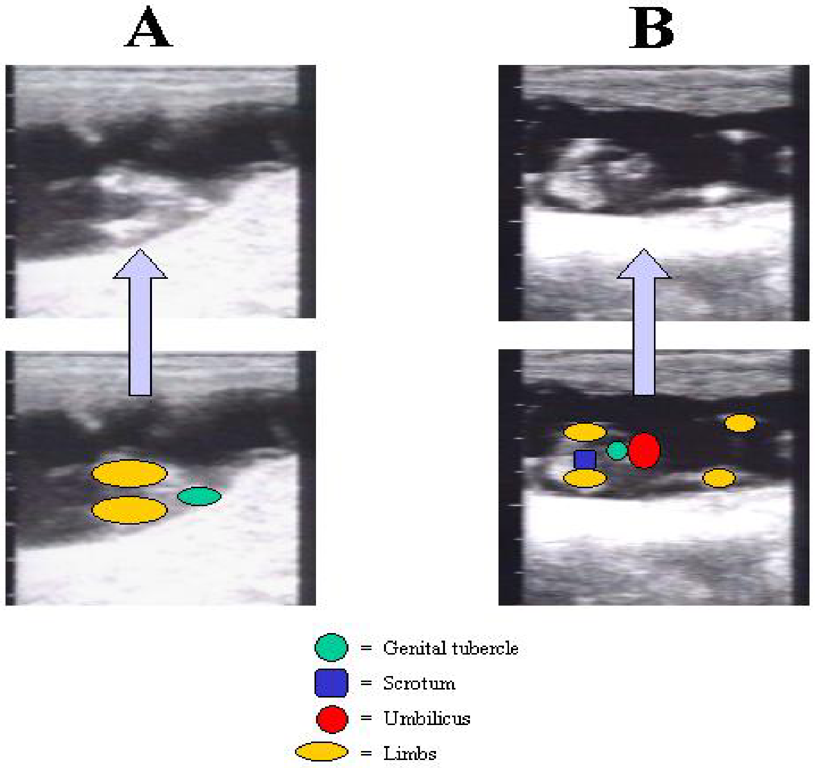

Figure 5. Ultrasound image of a female bovine fetus (65 days of gestation; Panel A) and a saggital view of a male fetus (65 days of gestation; Panel B). Images were taken using a 5.0 Mhz transducer.

Conclusion

The impact of real-time ultrasound on the study of reproduction has been dramatic and the further development of portable ultrasound machines has given clinicians an added tool for diagnostic reproductive management. Ultrasound is commonly used to monitor uterine anatomy, involution, and pathology. In addition, it has been used to detect pregnancy, study embryonic mortality, monitor fetal development, and determine fetal sex. The applications of ultrasound used by scientists include the ability to monitor follicular characteristics, ovarian function, and aid in follicular aspirations and oocyte retrieval. In the future, as technology improves technicians will have an opportunity to use the internet or video conferencing for ultrasound image analyses. With every new technological development, scientists, veterinarians, and producers discover new possibilities for the use of reproductive ultrasound to enhance the scientific merit of research or improve reproductive efficiency in cattle operations.

REFERENCES

Archibald LF, Thatcher WW, 1992. Ovarian follicular dynamics and management of ovarian cysts. In: Large Dairy Herd Management. Van Horn HH, Wilcox CJ, eds. Am Dairy Sci Assoc, Champaign, IL.

Aslan S, Findik M, Erunal-Maral N, Kalender H, Celebi M, Saban E. 2000. Comparison of various examination methods used in ovarian diagnostics in cattle. Dtsch. Tierarztl. Wochenschr. 107:227-230.

Badtram GA, Gaines JD, Thomas CB, Bosu WTK. 1991. Factors influencing the accuracy of early pregnancy detection in cattle by real-time ultrasound scanning of the uterus. Theriogenology 35:1153-1167.

Bartolome JA, Archibald LF, Morresey P. Hernandez J, Tran T, Kelbert D, Long K, Risco CA, Thatcher WW. 2000. Comparison of synchronization of ovulation and induction of estrus as theraputic strategies for bovine ovarian cysts in the dairy cow. Theriogenology 53:815-825.

Battocchio M, Gabai G, Mollo A, Veronesi MC, Soldano F, Bono G, Cairoli F. 1999. Agreement between ultrasonographic classification of the CL and plasma progesterone concentration in dairy cows. Theriogenology 51:1059-1069.

Beal WE, Perry RC and Corah LR. 1992. The use of ultrasound in monitoring reproductive physiology of beef cattle. J. Anim. Sci. 70:924-929.

Beghelli V, Boiti C, Parmigiani E, Barbacini S. 1986. Pregnancy diagnosis and embryonic mortality in the cow. In: J.M. Sreenan and M.G. Diskin(Ed.) Embryonic Mortality in Farm Animals. pp 159-167. Martinus Nijhoff, Boston.

Berry SL, Ahmadi A, Thurmond MC. 1994. Periparturient disease on large, dry lot dairies: interrelationships of lactation, dystocia, calf number, calf mortality, and calf sex. J Dairy Sci 77(Suppl 1):379.

Bierschwal CJ, Garverick HA, Martin CE, Youngquist RS, Cantley TC, Brown MD. 1975. Clinical response of dairy cows with ovarian cysts to GnRH. J Anim Sci 41:1660.

Boyd J, Bacisch P, Young A, McCracken JA. 1969. Fertilization and embryonic survival in dairy cattle. Br. Vet. J. 125:87-97.

Boyd JS, Omran SN, Ayliffe TR. 1988. Use of a high frequency transducer with real time B-mode ultrasound scanning to identify early pregnancy in cows. Vet. Rec. 123:8-11

Butler WR, Smith RD. 1989. Interrelationships between energy balance and postpartum reproductive function in diary cattle. J. Dairy Sci. 72:767-783.

Butler WR, Cherney DJR, Elrod CC. 1995. Milk urea nitrogen (MUN) analysis: Field trial results on conception rates and dietary inputs. Proc. Cornell Nutr. Conf. p 89.

Britt JH. 1992. Impacts of early postpartum metabolism on follicular development and fertility. Proc Annu Conv Am Assoc Bov Pract 24:39-43.

Britt JS, Gaska J, 1998. Comparison of two estrus synchronization programs in a large, confinement-housed dairy herd. JAVMA 212:210-212.

Curran S, Pierson RA, Ginther OJ. 1986. Ultrasonographic appearance of the bovine conceptus from days 20 through 60. JAVMA 189:1295-1302.

Curran S, Kastelic JP, Ginther OJ, 1989. Determining sex of the bovine fetus by ultrasonic assessment of the relative location of the genital tubercle. Anim Reprod Sci 19:217-227.

Davis ME, Haibel GK. 1993. Use of real-time ultrasound to identify multiple fetuses in beef cattle. Theriogenology 40:373-382.

Dawson FLM. 1975. Accuracy of rectal palpation in the diagnosis of ovarian function in the cow. Vet Rec 96:218.

Diskin, M.G. and J.M. Sreenan. 1980. Fertilization and embryonic mortality rates in beef heifers after artificial insemination. J. Reprod. Fertil. 59:463-468.

Erb RE, Morrison RA. 1959. Effects of twinning on reproductive efficiency in a Holstein-Fresian herd. J Dairy Sci 42:512-519.

Farin PW, Youngquist RS, Parfet JR, Garverick HA. 1990. Diagnosis of follicular cysts in dairy cows by sector scan ultrasonography. Theriogenology 34:636.

Fricke PM, Guenther, JN, Wiltbank MC, 1998. Efficacy of decreasing the dose of GnRH used in a protocol for synchronization of ovulation and timed AI in lactating dairy cows. Theriogenology 50:1275-1284.

Galland JC, Offenbach LA, Spire MF. 1994. Measuring the time needed to confirm fetal age in beef heifers using ultrasonographic examination. Vet Med 89:795-804.

Garverick HA, 1997. Ovarian follicular cysts in dairy cows. J Dairy Sci 80:995-1004.

Ginther, O.J. 1986. Ultrasonic imaging and reproductive event in the mare. Cross Plains, WI, Equiservices, Inc. pp 1-12.

Ginther OJ. 1995. Ultrasonic imaging and animal reproduction. Equiservices Publishing, Cross Plains, WI.

Ginther OJ, Wiltbank MC, Fricke PM, Gibbons JR, Kot K, 1996. Minireview. Selection of the dominant follicle in cattle. Biol Reprod 55:1187-1194.

Hansen PJ, Thatcher WW, Ealy AD. 1992. Methods for reducing effects of heat stress on pregnancy. In: VanHorn HH, Wilcox CJ (eds), Large Dairy Herd Management. Champaign IL: American Dairy Science Association 116-125.

Hanzen C, Delsaux B. 1987. Use of transrectal B-mode ultrasound imaging in bovine pregnancy diagnosis. Vet. Rec. 121:200-202.

Hughes EA, Davies DAR. 1989. Practical uses of ultrasound in early pregnancy in cattle. Vet. Rec. 124:456-458.

Jost A. 1971. Embryonic sexual differentiation. In: H.W. Jones and W.W. Scott (Ed.) Hermaphroditism, Genital Anomalies and Related Endocrine Disorders (2nd Ed.) pp 16-67. Williams and Wilkin, Baltimore, MD.

Kastelic JP, Bergfelt DR, Ginther OJ. 1990a. Relationship between ultrasonic assessment of the corpus luteum and plasma progesterone concentrations in heifers. Theriogenology 33:1269-1278.

Kastelic, J.P. and O.J. Ginther. 1989. Fate of conceptus and corpus luteum after induced embryonic loss in heifers. J. Am. Vet. Med. Assoc. 194:922-928.

Kastelic JP, Pierson RA, Ginther OJ. 1990b. Ultrasonic morphology of corpora lutea and central luteal cavities during the estrous cycle and early pregnancy in heifers. Theriogenology 34:487-498.

Kastelic JP, Bergfelt DR, Ginther OJ. 1991. Ultrasonic detection of the conceptus and characterization of intrauterine fluid on days 10 to 22 in heifers. Theriogenology 35:569-581.

Lamb GC. 2001. Reproductive Real-Time Ultrasound Technology: An Application for Improving Calf Crop in Cattle Operations. *In: Factors Affecting Calf Crop: Biotechnology of Reproduction. Ed. M.J. Fields*. pp 231-153. CRC Press LLC. Boca Raton. FL.

Lamb, G.C., B.L. Miller, V. Traffas, and L.R. Corah. 1997. Estrus detection, first service conception, and embryonic death in beef heifers synchronized with MGA and prostaglandin. *Kansas AES Report of progress* 783:97.

Lucy MC, Savio JD, Badinga L, De La Sota RL, Thatcher WW. 1992. Factors that affect ovarian follicular dynamics in cattle. J Anim Sci 70:3615-3626.

Lussier JG, Matton P, Dufour JJ. 1987. Growth rates of follicles in the ovary of the cow. J Reprod Fertil 81:301-307.

Mee JF, Ryan DP, Condon T. 1994. Ultrasound diagnosis of pregnancy in cattle. Vet Rec 134:532.

Morbeck DE, Esbenshade KL, Flowers WL, Britt JH. 1992. Kinetics of follicle growth in the prepubertal gilt. Biol Reprod 47:485-491.

Müller E, Wittkowski G. 1986. Visualization of male and female characteristics of bovine fetuses by real-time ultrasonics. Theriogenology 25:571(abstr.)

Murphy MG, Enright WJ, Crowe MA, McConnell K, Spicer LJ, Boland MP, Roche JF. 1991. Effect of dietary intake on pattern of growth of dominant follicles during the oestrous cycle in beef heifers. J Reprod Fertil 92:333-338.

Nanda AS, Ward WR, Dobson H, 1988. Retrospective analysis of the efficacy of different hormone treatments of cystic ovarian disease in cattle. Vet Rec 122:155.

Nebel RL, McGilliard ML. 1993. Interactions of high milk yield and reproductive performance in dairy cows. J. Dairy Sci. 76:3257-3268.

Paisley LG, Mickelsen WD, Frost OL, 1978. A survey of the incidence of prenatal mortality in cattle following pregnancy diagnosis by rectal palpation. Theriogenology 9:481.

Pfau KO, Bartlett JW, Shuart CE. 1948. A study of multiple births in a Holstein-Friesian herd. J Dairy Sci 31:241-254.

Pierson RA, Ginther OJ. 1984. Ultrasonography for detection of pregnancy and study of embryonic development in heifers. Theriogenology 22:225-233.

Pierson RA, Ginther OJ. 1988. Ultrasonic imaging of the ovaries and uterus in cattle. Theriogenology 29:21-37.

Pierson RA, Ginther OJ. 1988. Follicular populations during the estrous cycle in heifers III. Time of selection of ovulatory follicle. Anim. Reprod. Sci. 16:81-95.

Pieterse MC, Taverne MA, Kruip TA, Willemse AH, 1990. Detection of corpora lutea and follicles in cows: a comparison of transvaginal ultrasonography and rectal palpation. Vet Rec 156:552-554.

Pursley JR, Mee MO, Wiltbank MC. 1995. Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology 44:915-923.

Seguin BE, Convey EM, Oxender WD. 1976. Effect of gonadotropin-releasing hormone and human chorionic gonadotropin on cows with ovarian follicular cysts. Am J Vet Res 37:153-157.

Singh J, Pierson RA, Adams GP, 1997. Ultrasound image attributes of the bovine corpus luteum: structural and functional correlates. J Reprod Fertil 109:35-44.

Smith MW, Stevenson JS, 1995. Fate of the dominant follicle, embryonal survival, and pregnancy rates in dairy cattle treated with prostaglandin F2α and progestins in the absence or presence of a functional corpus luteum. J Anim Sci 73:3743-3751.

Spell AR, Beal WE, Corah LR, Lamb GC. 2001. Evaluating recipient and embryo factors that affect pregnancy rates of embryo transfer in beef cattle. Theriogenology 56:287-298.

Spicer LJ, Echternkamp SE, 1986. Ovarian follicular growth, function and turnover in cattle: a review. J Anim Sci 62:428-451.

Stevenson JS, Schmidt MK, Call EP. 1984. Stage of estrous cycle, time if insemination, and seasonal effects on estrus and fertility of Holstein heifers after prostaglandin $F_{2\alpha}$. J. Dairy Sci. 67:1798-1805.

Szenci O, Beckers JF, Humblot P, Sulon J, Sasser G, Taverne MA, Varga J, Baltusen R, Schekk G. 1998. Comparison of ultrasonography, bovine pregnancy-specific protein B, and bovine pregnancy-associated glycoprotein 1 tests for pregnancy detection in dairy cows. Theriogenology 50:77-88.

Szenci O, Gyulai G, Nagy P, Kovacs L, Varga J, Taverne MAM. 1995. Effect of uterus position relative the pelvic inlet on the accuracy of early bovine pregnancy diagnosis by means of ultrasonography. Vet Quarterly 17:37-39.

Taverne MAM, Szenci O, Szetag J, Piros A. 1985. Pregnancy diagnosis in cows with linear-array real-time ultrasound scanning: a preliminary note. Vet. Quarterly 7:264-270.

Vaillancourt D, Vierschwal CJ, Ogwu D, Elmore RG, Martin CE, Sharp AJ, Youngquist RS, 1979. Correlation between pregnancy diagnosis by membrane slip and embryonic mortality. J Am Vet Med Assoc 175:466.

Vasconcelos JLM, Silcox RW, Lacerda JA, Pursley JR, Wiltbank MC, 1997. Pregnancy rate, pregnancy loss, and response to heat stress after AI at 2 different times from ovulation in dairy cows. Biol Reprod 56(Suppl 1):140 abstr.

Wideman D, Dorn CG, Kraemer DC. 1989. Sex detection of the bovine fetus using linear array real-time ultrasonography. Theriogenology 31:272(abstr.).

Whitmore HL, Hurtgen JP, Mather EC, Seguin BE, 1979. Clinical response of dairy cattle with ovarian cysts to single or repeated treatments of gonadotropin-releasing hormone. JAVMA 174:1113-1115.

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