# PRACTICAL APPLICATIONS OF ULTRASOUND FOR REPRODUCTIVE MANAGEMENT OF DAIRY CATTLE

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## INTRODUCTION

Application of transrectal real-time ultrasonography to the study of bovine reproduction represents a technological breakthrough that has revolutionized our knowledge of reproductive biology. New research information generated through ultrasonic imaging has clarified the nature of complex reproductive processes in cattle including ovarian follicular dynamics, corpus luteum function, and fetal development. Widespread adoption and use of ultrasonography for routine reproductive examinations of dairy cattle is the next contribution this technology will make to the dairy 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).

Assessment of pregnancy status and fetal viability early post breeding to identify cows that fail to conceive improves reproductive efficiency by decreasing the interval between AI services and increasing AI service rate. Early identification of cows carrying twin fetuses allows for implementation of differential management strategies to abrogate negative effects of twinning during the periparturient period. Ovarian and uterine pathologies not accurately detected via rectal palpation can easily be visualized and appropriate therapies selected and implemented. Determination of fetal sex in utero is useful when coupled with a management decision that justifies the expense of fetal sexing. Development of integrated reproductive management systems that combine ultrasound with new and existing reproductive technologies will further ehnance the practical applications of ultrasonography. Collectively, current and future applications of ultrasonography hold tremendous potential to enhance reproductive management and improve reproductive efficiency in dairy cattle. Development of Extension education programs to train bovine practitioners to use ultrasound for routine reproductive examinations is a critical step toward rapid implementation of this technology into the dairy industry. As ultrasound equipment becomes increasingly portable and less costly, it is only a matter of time until widespread implementation of this technology occurs in the dairy industry.

## VETERINARY ULTRASOUND EQUIPMENT

Detailed information on the principles of ultrasonography is beyond the scope of this review and can be found elsewhere (Ginther, 1995). In general, linear-array, real-time, B-mode ultrasound scanners are best suited for veterinary applications involving dairy 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 a dairy operation. 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 in free-stall barns 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 in dairy operations. The author's opinion is that it is not a matter of *if* this transition will occur, but *when* it will occur.

## IMAGING THE BOVINE OVARY

### Ovarian Structures as Diagnostic Aids

Routine reproductive examinations in dairy cattle 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 noninvasively track follicular growth during the estrous cycle using ultrasound has revolutionized our understanding of reproductive physiology.

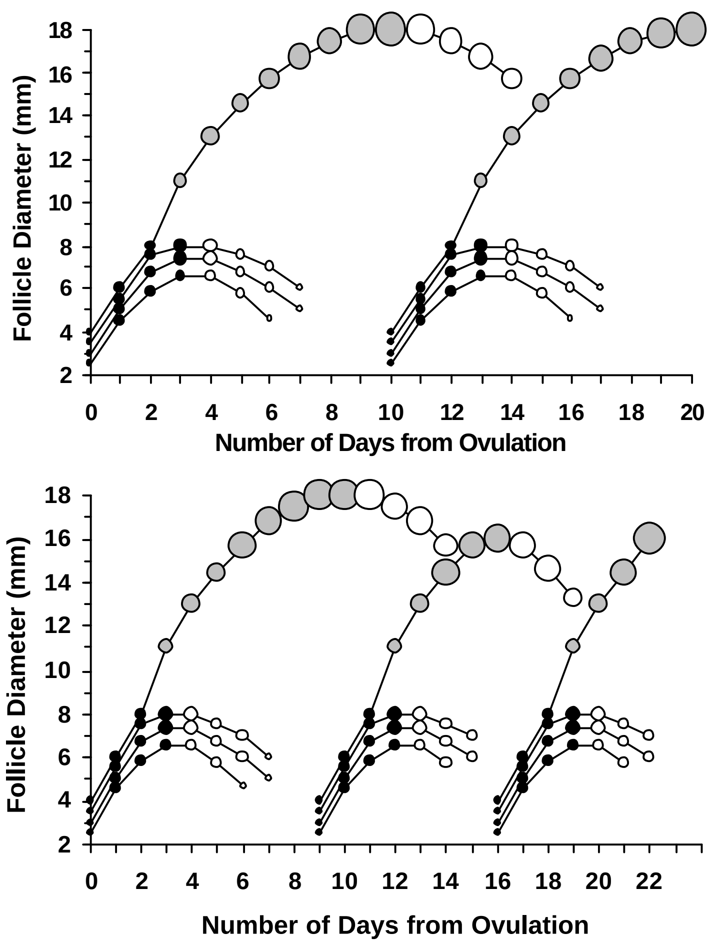


**Figure 1.** Schematic diagram depicting two-wave (top panel) and three-wave (bottom panel) patterns of follicular growth during the bovine estrous cycle. Growing follicles before selection of the dominant follicle are depicted as black circles, the dominant follicles of each wave are depicted as gray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

Under normal circumstances, follicular waves ensure that only one follicle capable of undergoing ovulation is present at any given time during the estrous cycle. In general, primiparous and multiparous lactating dairy cows exhibit more two-wave cycles, whereas nulliparous dairy heifers tend to exhibit more three-wave and four-wave cycles (unpublished obaservations). Some factors that may influence the number of waves per estrous cycle in dairy cattle include dietary intake (Murphy et al., 1991) age, parity, and lactational status (Lucy et al., 1992).

## 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). Ovarian cysts containing luteinized tissue (discussed in the following section) should not be confused with normal CL containing a fluid-filled cavity.

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 lactating cows. 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

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 is an effective treatment 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, however, 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

Detection of the embryo proper as well embryonic and fetal developmental characteristics during early fetal development are shown in Table 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 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 |

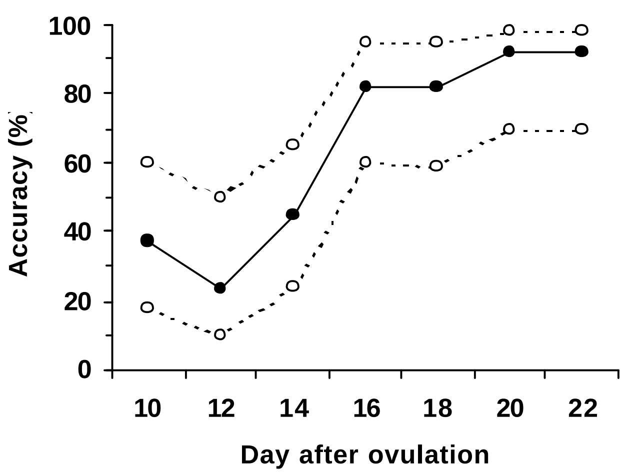


**Figure 2.** Mean (and 95% confidence interval) accuracy of early pregnancy diagnosis using transrectal ultrasonography in dairy heifers (Adapted from Kastelic et al., 1991).

### Early Pregnancy Diagnosis

The use of transrectal ultrasonography to assess pregnancy status early during gestation is among the most practical applications of ultrasound for dairy cattle reproduction. Early identification of open cows post breeding improves reproductive efficiency and pregnancy rate in a dairy by decreasing the interval between AI services and increasing AI service rate. Pregnancy diagnosis in dairy heifers based on the presence of intraluminal uterine fluid before Day 16 is unreliable because small amounts of fluid are present in non-inseminated heifers as early as Day 10; however, accuracy of diagnosis based on fluid alone approached 100% by Day 20 (Figure 2). The accuracy of pregnancy diagnosis in dairy heifers was not greater than 50% before Day 18 using a 5.0 MHz transducer, or before Day 16 using a 7.5 MHz transducer (Kastelic et al., 1991). Although pregnancy status can be established early, care must be taken to ensure the accuracy of a diagnosis. For example, a false negative diagnosis was more likely when the uterus was located cranial to the pelvic inlet in beef cattle than when the uterus was within the pelvic cavity (Szenci et al., 1995).

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 dairy 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. Unfortunately, the service rate was only 58% when using a system combining $PGF_{2\alpha}$ and Kamar heat mount detectors (Britt and Gaska, 1998), probably due to the inherent inefficiencies of estrus expression and detection in lactating dairy cows.

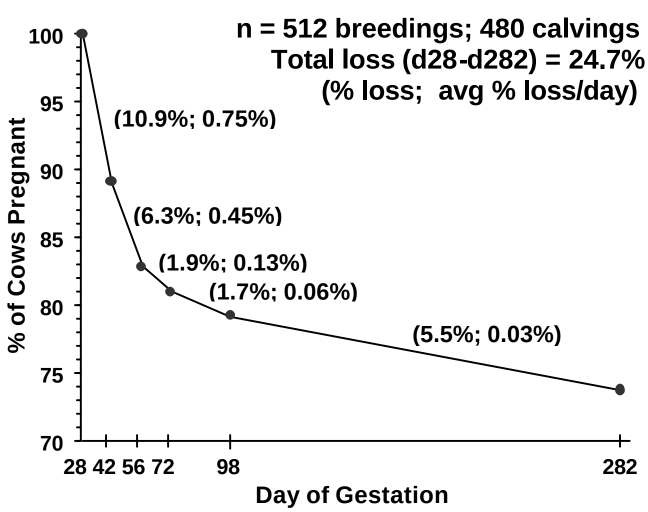


**Figure 3.** Pregnancy losses from 28 days post AI to calving in lactating dairy cows. Pregnancy status was diagnosed using ultrasound at 28, 42, 56, 70, and 98 days post AI, and calving data were recorded at parturition. The conception rate at 28 days was 32%. Data adapted from Vasconcelos et al., 1997.

## Early Embryonic Loss

Pregnancy loss contributes to reproductive inefficiency because fertility assessed at any point during pregnancy is a function of both conception rate and pregnancy loss. Conception rates at 28 to 32 days post-AI in lactating dairy cows range from 40 to 47% (Pursley et al, 1997; Fricke et al., 1998), whereas conception rates in dairy heifers are nearly 75% (Pursley et al., 1997). Similarly, pregnancy loss in lactating dairy cows is greater than that in dairy heifers (20% vs. 5%; Smith and Stevenson, 1995). Although the specific factors responsible for early embryonic loss in dairy cows are not known, they may be similar to those factors responsible for reduced conception rates.

Because pregnancy status can be determined earlier using ultrasound than palpation, the rate of pregnancy loss detected is often higher. Of cows diagnosed pregnant at 28 days post AI, 14 to 10 to 16% experience early embryonic loss by 56 days post AI (Mee et al., 1994; Vasconcelos et al., 1997; Fricke et al., 1998). Therefore, cows diagnosed pregnant at 28 days post AI using ultrasound should be scheduled for reexamination around 60 days post AI, when the rate of embryonic loss per day decreases dramatically (Figure 3). Although the rate of pregnancy loss is significant in studies using ultrasound to assess pregnancy loss, the technique of ultrasound itself has not been implicated as a cause of embryonic death in cattle (Ball and Logue, 1994; Baxter and Ward, 1997). Furthermore, ultrasound is a much less invasive technique for early pregnancy diagnosis than is rectal palpation (Paisley et al., 1978; Vaillancourt et al., 1979) and may minimize the rare incidence of palpation-induced abortions.

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.

## Identification of Cows Carrying Twins

Twinning is an unavoidable outcome of reproduction in dairy cattle and is undesirable in a dairy operation because it reduces overall profitability and reproductive efficiency (Eddy et al., 1991; Beerepoot et al., 1992). The reported incidence of twinning in dairy cattle ranges from 2.5 to 5.8% and is dramatically affected by parity, ranging from 1% for first parity to nearly 10% during later parities (Table 3).

**Table 3.** Effect of parity on twinning rate (%) in dairy cattle.

| | No. of | Parity | | | | | | All |
|---|---|---|---|---|---|---|---|---|
| Ref[a] | Calvings | 1 | 2 | 3 | 4 | 5 | 6 | Parities |
| 1 | 937 | 0.7 | 5.0 | 4.2 | 5.0 | 7.0 | 6.7[b] | 4.2 |
| 2 | 7,387 | 1.3 | 4.4 | 5.3 | 4.6 | 5.8 | 6.0 | 4.6 |
| 3 | 11,951 | 0.8 | 2.7 | 4.1 | 4.5 | 4.9 | 4.8[b] | 3.2 |
| 4 | 19,755 | 0.9 | 2.1 | 3.5 | 3.4 | 3.7 | 3.2 | 2.5 |
| 5 | 24,843 | 1.0 | 7.0 | 7.5 | 7.9 | 9.1[b] | - | 4.2 |
| 6 | 19,497 | 1.3 | 6.0 | 9.4[b] | - | - | - | - |
| 7 | 52,362 | 1.0 | 2.9 | 3.2 | 3.9 | 3.3 | 4.1[b] | 2.4 |

[a]Reference: 1=Pfau et al., 1948; 2=Erb & Morrison, 1959; 3=Nielen et al., 1989; 4=Eddy et al., 1991; 5=Ryan & Boland, 1991; 6=Berry et al., 1994; 7=Kinsel et al., 1998.

[b]Includes all cows ≥ the parity listed.

The effect of parity on twinning rate is not clearly understood but may be explained by an increased ability of older cows to support twins throughout gestation, an increase in the rate of double ovulation, or an interaction of both of these factors. Increased uterine capacity of cows calving twins has been reported (Ryan and Boland, 1991). Furthermore, the incidence of double ovulation in lactating dairy cows is around 14% (Kidder et al., 1952; Fricke et al., 1998), and, as with the incidence of twinning, increases with parity (Labhsetwar et al., 1963; Fricke and Wiltbank, 1999). Kinsel et al. (1998) reported an increase rate of twinning over a 10-year period.

The single largest related factor to this increase was the increase in peak milk production that occurred over that period. They also suggested that feeding higher energy diets to high producing cows may be increasing the incidence of double ovulations, and hence the rate of twinning. We recently reported a relationship between milk production and the incidence of double ovulation in lactating dairy cows (Table 4).

The overall twinning rates reported for dairy cows in recent studies are greater than those reported in many earlier reports (Day et al., 1995), indicating that twinning rate may be increasing over time in the dairy cattle population as a whole. If twinning is related to nutrition and/or milk production, this increase in twinning would not be unexpected considering recent trends in feeding practices and yearly increases in milk production per cow.

**Table 4.** Effect of parity and milk production (mean ± SEM) on the incidence of double ovulation after a synchronized ovulation (adapted from Fricke and Wiltbank, 1999).

| | **Milk production (kg/d)** | | |
|---|---|---|---|
| **Item** | **Low (£40)** | **High (>40)** | **Overall** |
| Milk production (kg/d) | 31.1 ± 0.7 | 50.7 ± 0.7 | 40.5 ± 0.8 |
| Parity 1 (%) | 7.4 | 22.2 | 9.5[c] |
| Parity 2 (%) | 4.0 | 14.3 | 10.8[d] |
| Parity 3 (%) | 8.7 | 27.8 | 20.3[e] |
| Overall (%) | 6.9[a] | 20.2[b] | 13.3 |

[a,b]Proportions differ by chi-square analysis ($p$<0.05).
[c,d,e]Linear increase ($p$=0.09) in incidence of double ovulation with increasing parity.

Cows carrying twin pregnancies can be accurately identified using transrectal ultrasonography by 40 to 55 days post AI (Echternkamp and Gregory, 1991; Davis and Haibel, 1993; Dobson et al., 1993). When conducting an early diagnosis for twins, the entire length of both uterine horns must be carefully scanned to ensure that an embryo is not missed. Because the majority of twinning in dairy cattle occurs due to double ovulations (Wiltbank et al., In press), the presence of two or more CL on the ovaries at the time of diagnosis can be an excellent indicator of cows with an increased risk of carrying twins. In general, ovarian indicators such as number and size of CL can enhance diagnosis of pregnancy status and twinning.

**Table 5.** Estimated intervals and cumulative days in milk associated with events after a management decision to terminate a pregnancy after diagnosis of twins.

| **Mean interval from:** | **Interval (Days)** | **Cumulative Days in Milk** |
|---|---|---|
| Calving to twin pregnancy diagnosis and induction of abortion | 184[a] | 184 |
| Induction of abortion to second conception | 84[b] | 268 |
| Second conception to dry off | 232[c] | 500 |

[a]Average days open (144 days; voluntary waiting period = 60 days, conception rate = 40%, and service rate = 40%)+ day of gestation at diagnosis of twins (40 days).
[b]Median days to second conception using AI breeding (84 days; conception rate = 40%, and service rate = 40%).
[c]Average gestation length (282 days) - average dry period (50 days).

Several management scenarios could be considered upon identification of a cow carrying twins. Continued management of the cow could be avoided either by culling the cow or by aborting the twin pregnancy, usually through administration of an ecbolic agent such as $PGF_{2\alpha}$.

Several factors would argue against aborting a twin pregnancy with the intent of rebreeding the cow. First, the estimated average lactation length of cows subjected to induced abortion and rebreeding would approach 500 days (~18.5 month calving interval) based on average reproductive performance and management indices for lactating cows (Table 5). Second, the risk for a twin pregnancy during the subsequent gestation is increased because cows calving twins are at greater risk for subsequent twinning (Nielen et al., 1989). Based on these considerations and depending on the value of the dam and calf, culling may be a better alternative to aborting the pregnancy.

## Fetal Sexing

Transrectal ultrasound can be used to detect the sex of bovine fetuses *in vivo*. Sex is determined by evaluating the morphology of the genital tubercle using ultrasound and is a reliable and accurate method for sex determination beginning on Day 55 to 60 of gestation (Curran et al., 1989). Because the reproductive tract and conceptus descend beyond the pelvic rim and into the abdominal cavity as gestation ensues, it becomes increasingly difficult to reach the fetus and make an accurate diagnosis during later stages of gestation. Generally, a greater level of operator experience and proficiency is required for sex determination using ultrasound compared with that required for early pregnancy diagnosis or examination of ovarian structures.

Determination of fetal sex is useful when combined with a management decision or strategy that justifies the expense of fetal sexing. In other words, a producer who pays for information regarding fetal sex must economically justify the usefulness of that information. Filling sales contract obligations regarding the sex of a calf carried by a pregnant cow to be sold is one scenario that may justify this expense. If the sex of a calf is a determining factor for culling decisions regarding a pregnant cow, fetal sexing might be justified. In contrast, the cost associated with fetal sexing is unwarranted if the information is not used to make a management decision. Because of the economic and management considerations associated with fetal sexing, routine fetal sexing of all pregnant cow in a dairy herd will not likely become a standard reproductive management practice on most dairies.

## Reproductive Management Strategies

Figure 4 shows a scenario for combining use of Ovsynch and early pregnancy diagnosis using ultrasound. Groups of cows past the voluntary waiting period would receive their first postpartum insemination after synchronization of ovulation using Ovsynch. This would dramatically reduce median days to first AI by eliminating estrus detection for the first postpartum breeding. On Day 25 post AI, ultrasound would be used to identify nonpregnant cows, which would receive the first GnRH injection for resynchronization using Ovsynch. This would result in an average interval between services of 35 days for cows requiring resynchronization.


**Figure 4.** Reproductive management protocol for combining timed AI (Ovsynch) with early pregnancy diagnosis using ultrasound. Note that hormone injections are scheduled for Monday (M) and Wednesday (W), ultrasound examinations for Monday (M), and timed AI (TAI) for Thursday (T). Average interval between services would be 35 days for cows requiring resynchronization.

Figure 5 shows a more aggressive scenario for combining use of Ovsynch and early pregnancy diagnosis using ultrasound. Groups of cows past the voluntary waiting period would receive their first postpartum insemination after synchronization of ovulation using Ovsynch. On Day 18 post AI, all cows would receive an injection of GnRH regardless of their pregnancy status. Ultrasound would be used on Day 25 to identify nonpregnant cows, which would receive a PGF$_{2\alpha}$ injection for resynchronization using Ovsynch. Although recent data has suggested that administration of GnRH to pregnant cows may increase early embryonic loss (Moreira et al., 2000), these data have not been replicated. Further research into the efficacy of such protocols combining timed AI with ultrasonography for reproductive management of dairy cattle is ongoing.

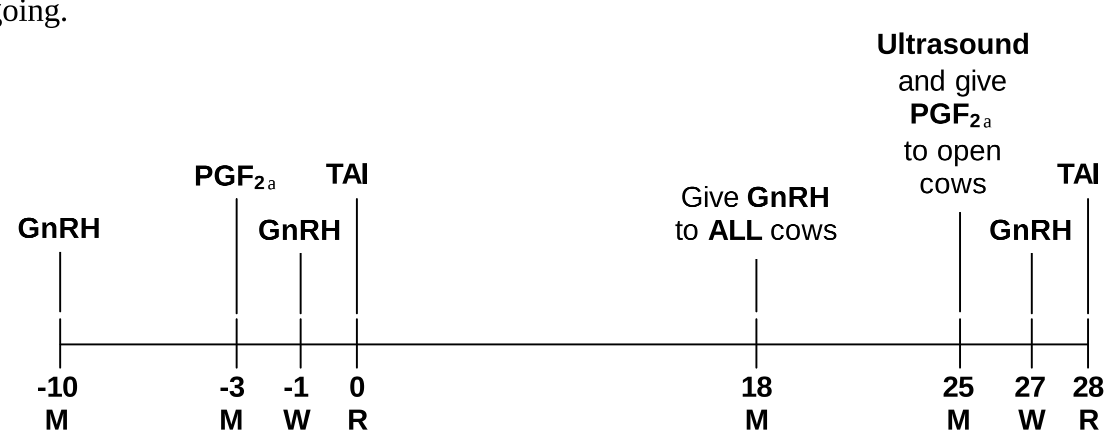


**Figure 5.** Aggressive reproductive management protocol for combining timed AI (Ovsynch) with early pregnancy diagnosis using ultrasound. Note that hormone injections are scheduled for Monday (M) and Wednesday (W), ultrasound examinations for Monday (M), and timed AI (TAI) for Thursday (T). Average interval between services would be 28 days for cows requiring resynchronization.

## CONCLUSION

As a research tool, transrectal ultrasound has revolutionized our understanding of reproductive biology. As a management tool, transrectal ultrasound will provide a diagnostic tool for improving reproductive management in dairy operations. Although there are many potential applications of ultrasound for use in reproduction, combining ultrasound for early pregnancy diagnosis with timed AI along with early detection of twin pregnancies will likely result in the most widespread uses of this technology.

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