

# Reproductive Applications of Ultrasound in the Cow

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## Abstract

During the past decade portable ultrasound units that provide excellent image quality have become available to veterinary practitioners. The use of transrectal ultrasonography for evaluation of reproductive structures in cattle extends and enhances the diagnostic capabilities of practitioners beyond the traditional use of palpation *per rectum*. Ultrasonography of ovaries enables more consistent differentiation of ovarian follicles and corpora lutea. Accurate counting of follicles and precise measurement of follicles or corpora lutea is possible. Sequential monitoring of ovarian structures enables the user to characterize ovarian follicular waves, to identify dominant follicles and to detect ovulation. Images can be used to identify, objectively measure and to characterize the quality of a corpus luteum. Ultrasonography is also a valuable tool for identification of abnormal (cystic) luteal and follicular structures. Pregnancy diagnosis can be performed using ultrasound by day 19 to 24 post-breeding and fetal viability can be verified by visualization of a fetal heartbeat. Aging embryos and fetuses between days 20 and 100 of gestation can be facilitated by measuring crown-rump length (20-50 days) or the diameter of the head or trunk (50-100 days). Sexing of bovine fetuses is accomplished by visualizing the genital tubercle (penis) and scrotum in males or by the lack of male genitalia and the presence of the genital tubercle (vulva) in females. Sex determination is most practical between days 60 and 85 of gestation. Like other diagnostic tools, ultrasound has limitations. Skill of the ultrasound technician in capturing clear images, differentiating anatomical orientations and interpreting ultrasound images is essential. The effective use of ultrasound technology enhances diagnostic capabilities and increases the services offered by veterinarians.

## Résumé

Durant la dernière décennie, les appareils portables à ultrasons offrant des images d'excellentes

qualités sont devenus disponibles auprès des vétérinaires praticiens. L'utilisation transrectale de l'ultrasonographie pour l'examen des structures reproductives chez les bovins a permis d'accroître les capacités diagnostiques des praticiens au-delà de la palpation rectale traditionnelle. L'ultrasonographie des ovaires permet une différenciation plus constante des follicules ovulaires et des corps jaunes. Un décompte adéquat des follicules de même que des mesures précises des follicules ou des corps jaunes est possible. Le suivi séquentiel des structures ovariennes permet à l'utilisateur de caractériser les vagues folliculaires ovariennes, d'identifier les follicules dominants et de détecter l'ovulation. Les images peuvent être utilisées pour identifier, mesurer objectivement et caractériser la qualité d'un corps jaune. L'ultrasonographie est aussi un outil précieux pour l'identification des structures lutéales ou folliculaires anormales (kystiques). Le diagnostic de gestation peut aussi se faire avec des ultrasons de 19 à 24 jours suivant la fécondation et la viabilité du fœtus peut être jugée suite à la visualisation du poulx cardiaque. La détermination de l'âge des embryons et des fœtus entre 20 et 100 jours de gestation est rendue plus facile par la mesure de la longueur entre la tête et la croupe (20-50 jours) ou du diamètre de la tête ou du tronc (50-100 jours). La détermination du sexe des fœtus bovins se fait suite à la visualisation du pénis et du scrotum chez les mâles et de la présence de la vulve chez les femelles. La détermination du sexe est possible surtout entre les jours 60 et 85 de la gestation. Comme les autres outils diagnostics, l'utilisation des ultrasons a ses limites. L'habileté du technicien est essentielle pour produire des images claires, pour différencier les orientations anatomiques et interpréter les images. L'utilisation appropriée des ultrasons accroît la capacité diagnostique et les services offerts par les vétérinaires.

## Introduction

The use of transrectal ultrasonography to evaluate the reproductive tract in cows has enhanced our understanding of ovarian and uterine processes during

both the bovine estrous cycle and pregnancy. Ultrasound has improved our ability to manipulate these processes in order to improve reproductive performance and increase genetic improvement of cattle. It has also provided a window to examine the environment of the fetus, to better understand the interaction between the fetus and its dam, and to accurately predict fetal sex. Ultrasonography has literally been an "eye-opening" experience, changing static glimpses that were achieved via palpation, laparoscopy or postmortem examination into real-time images. Although ultrasound is a powerful tool, the scanning images do not reveal the biochemical processes that govern reproductive function.

A principal reason for the increased routine use of ultrasound in cattle has been the development of relatively inexpensive, portable equipment. Several companies now offer excellent ultrasound units for diagnostic examination of large or small animals (Table 1).

The purpose of this article is to review the literature pertaining to reproductive ultrasound in the cow, as well as to share some practical lessons learned through the application of ultrasound for monitoring reproduction in cattle. The objective is to describe the use of ultrasound to monitor normal and pathological ovarian structures, to describe uterine characteristics during the estrous cycle, pregnancy and disease, and to describe monitoring of fetal viability and gender identification.

### Principles of Ultrasound

Real-time, B-mode ultrasound provides a display mode in which the signal echoed from a tissue is displayed as a dot. The intensity of the dot is proportional to the amplitude of the signal, and its position is relative to the distance between the transducer and the reflective tissue surface. The image is 2-dimensional,

created by rapid succession of B-mode traces so that as the ultrasound transducer moves, the image changes, depicting motion in real-time.

The ultrasound transducer acts to send and receive sound waves. When an electric field is applied to the piezoelectric crystals in the transducer, they change shape and vibrate, creating waves of sound. The ultrasound transducer directs these high frequency, low intensity sound waves toward the tissues. The crystals are arranged in a linear fashion along the face of most common transducers, hence, the name linear array. Different proportions of the sound waves emitted are reflected back to the transducer, depending on the density of the tissue. The returning sound waves produce pressure on the crystals, generating an electric charge that is converted to a visual image (dot) on the screen. Fluid, such as blood or follicular fluid, does not reflect sound waves; this no-image (black) appears on the screen. This is referred to as 'nonechogenic' or 'anechoic'. Bone is the densest tissue and reflects sound waves almost completely. The bright white image depicting the bone surface is referred to as 'hyper-echogenic' or 'hyperechoic'. Other tissues reflect varying proportions of sound waves and produce images of various shades of gray.

Differences in the reflection of sound waves from various tissues, or differences in the angle at which sound waves strike tissue surfaces, may cause echogenic artifacts. These artifacts appear either as a signal enhancement, a repetitive signal or a shadow (no signal). Because fluids do not impede the passage of sound, tissues that reflect waves after they pass unattenuated through a fluid compartment may appear denser than normal (hyperechogenic). This is referred to as enhancement artifact. This artifact can distort the appearance of tissues positioned below fluid-

**Table 1.** Major suppliers of ultrasound equipment.<sup>a</sup>

Ultrasound Supplier	Address	Telephone
Aloka	10 Fairfield Blvd. Wallingford, CT 06492	1-800-442-5652
Universal Medical Systems	299 Adams St. Bedford Hills, NY 10507	1-914-666-6200
Products Group International	P.O. Box 1807 Lyons, CO 80540	1-800-336-5299
Classic Medical Supply Inc.	19900 Mona Rd. Suite 105 Tequesta, FL 33469	1-561-746-9527
Alliance Medical, U.S.A.	112 N. Bridge St. P.O. Box 404 Smithville, MO 64089	1-816-532-4838

<sup>a</sup>Mention of suppliers does not imply endorsement nor does failure to mention a supplier imply a lack of endorsement of that corporation or its equipment.

filled structures. On the other hand, bone impedes the passage of sound waves almost completely and a shadow (nonechogenic) appears below bony structures making imaging of those tissues impossible. Shadowing is also caused when sound waves encounter a tangential surface such as the lateral margins of fluid-filled cavities. The beam distal to the margin is weaker due to the attenuation of the beams and a shadow appears. Reverberation or echoes occur when sound waves bounce between the transducer and the surfaces of the reflecting structures. Strong reflecting substances, such as metal or bone, can create a "comet-tail" effect due to reverberation. Mirror images may be produced when the sound waves encounter an air interface. Hence, reflected waves bounce off the transducer back to the interface and back again to the transducer. The ultrasound unit interprets this time lag of echoes as images beyond the air interface and reproduces the image on the other side of the air interface.

Understanding the principles and limitations of ultrasound technology enhances interpretation of ultrasound images. Successful monitoring of reproductive events using ultrasonography depends on the ability of the technician to capture a clear image with maximum resolution and the ability to interpret that image.

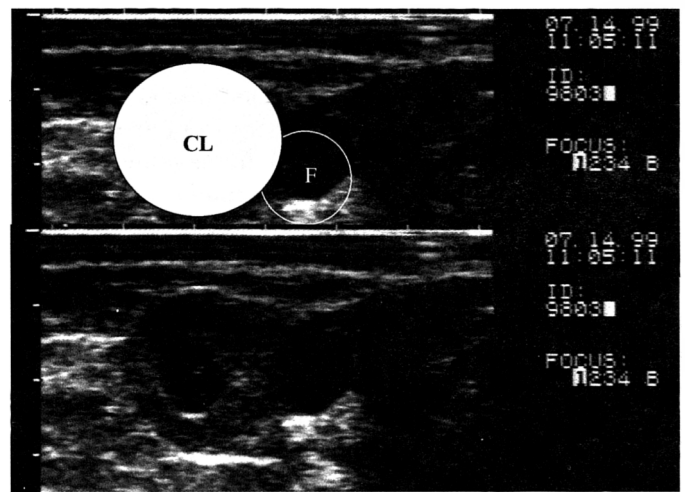
### Monitoring Ovarian Structures

Prior to ultrasound, evaluation of bovine ovarian follicles was limited to palpation, laparoscopy or visual examination of excised ovaries. With the advent of ultrasound, however, non-invasive, repeated monitoring of bovine follicular and luteal development became possible (Figure 1). Resolution and clarity of ovarian images depend on the quality of the ultrasound equipment and the experience of the operator.<sup>45</sup> However, ultrasound is a more accurate method than palpation *per rectum* for detecting and measuring ovarian follicles, especially those lying within the ovarian stroma.<sup>30,39</sup> Correlation coefficients between ultrasound measurements and actual measures obtained from slicing ovaries recovered following slaughter ranged from .80 to .92 for number of follicles detected in various size categories, and was .97 for diameter of the largest follicle.<sup>37</sup>

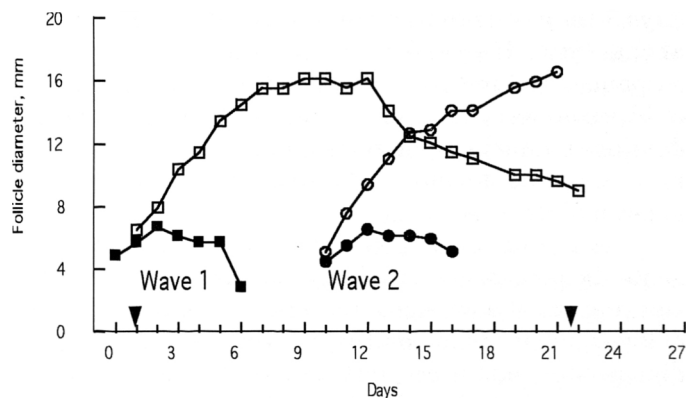
Ultrasonic monitoring of ovarian function has been used to determine that bovine follicular development occurs in two, three or four coordinated waves throughout the estrous cycle and that follicular waves continue to occur at approximately 10-day intervals during pregnancy (Figure 2). As several follicles begin development, one follicle emerges as the dominant follicle which suppresses the development of its cohorts.<sup>16,18,29,42</sup> A dominant follicle appears in each wave and continues to enlarge while suppressing the growth of subordinate follicles. If luteolysis is initiated while the dominant

follicle is still functional, the dominant follicle will begin secreting estrogen and ovulate. If the dominant follicle begins to regress before luteolysis is complete, another follicular wave will ensue.<sup>26</sup>

The echotexture characteristics of the dominant follicle are related to the functional and endocrine status of the follicle.<sup>41,47</sup> After the dominant follicle reaches its peak diameter (referred to as the static phase) granulosa cells are sloughed into the antrum. This debris increases the echogenic heterogeneity of antral fluid. The changes in follicular echotexture, as measured by computer-assisted echotexture analysis, coincide with both the ovulatory potential of the follicle and steroid content of follicular fluid.<sup>41,47</sup> At present, however, there is no method to determine the physi-



**Figure 1.** Ultrasound image of bovine ovary with a corpus luteum (CL) and follicle (F). Upper image has diagrammatic representations of structures in lower image.



**Figure 2.** Bovine estrous cycle characterized by two follicular waves (arrow indicates ovulation; open symbols = dominant follicle; filled symbols = subordinate follicles).

ological status of a large follicle without serial examinations and retrospective analysis. Future use of computer-assisted image analysis may improve the diagnostic potential of ultrasound to determine the status of a large follicle in a single examination.

The effects of nutrition and bovine somatotropin (bST) treatment on ovarian follicular development in lactating and non-lactating Holsteins have been examined with ultrasound.<sup>10,31</sup> Lactating cows fed low energy diets or treated with bST had increased numbers of smaller (<10 mm) follicles, larger subordinate follicles and higher plasma estradiol levels than non-lactating cows, possibly due to reduced health or secretory capacity of the dominant follicle.<sup>10,31</sup> The development of ovarian follicles in lactating cows treated with bST resembled that of non-lactating cows.<sup>10</sup> Correct classification of the follicular structure on the ovary requires knowledge of the type of cattle, stage of production, nutritional status, current treatments and ovarian effects and medical history of the patient. Discernment between conditions indicating treatment (persistent anovulatory follicles or cystic ovarian disease), management action (large, pre-ovulatory follicle) and normal ovarian structures (normal dominant follicles) is critical for proper veterinary care.<sup>49</sup>

Several studies have been conducted to test the effects of a dominant follicle present at the initiation of follicle stimulating hormone (FSH) treatment on the superovulatory response. Guilbault and coworkers<sup>17</sup> determined that ovulation rate was reduced by 40 to 50% and was more variable in dairy heifers treated with FSH while a dominant follicle was present. Dominant follicles were defined in that study as being >9mm in diameter and either in a growth or static phase. The removal of a dominant follicle was followed by an increase in circulating FSH and a subsequent increase in small follicles.<sup>1</sup> Heifers superovulated in the absence of a dominant follicle had more corpora lutea 7 days after estrus than heifers with a dominant follicle at that time. If an embryo transfer donor has failed to respond to a standard superovulation regimen, the use of ultrasonography to characterize the activity of the dominant follicle prior to beginning FSH treatment may assist in developing a strategy to improve the superovulatory response.

Ovulation, as detected by ultrasonography, is the acute disappearance of a large follicle (9-20 mm) that was present at a previous, recent examination. The site of ovulation is visible on the day that the large follicle disappears, and the corpus luteum (CL) may develop as either a solid or fluid-filled structure (as in Figure 1). The cavities of fluid-filled corpora lutea are distinguished from follicles by a non-spherical, often lobulated, appearance and by the surrounding border of luteal tissue. Several researchers have demonstrated that a CL with

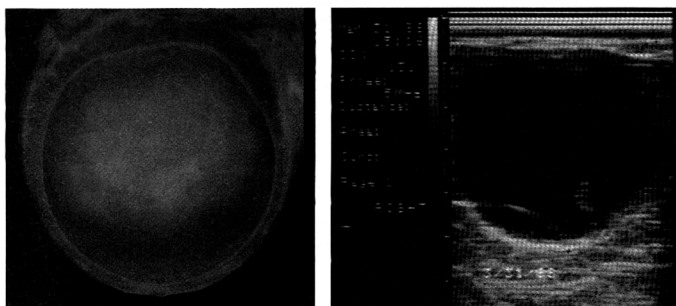
a fluid-filled cavity is a normal condition and that the cavity is usually replaced by a dense, solid core of luteal tissue late in the estrous cycle or during the first 25 days of pregnancy.<sup>38</sup>

The relative echogenicity of the corpus luteum (CL) depends on the stage of CL development. The corpus hemorrhagicum is visible from ovulation to day 3 post-ovulation as it is less dense than the surrounding stroma and often has an anechoic, fluid-filled center.<sup>35</sup> The corpus luteum can be detected more easily by 3 days after ovulation. The growth of the CL is most extensive between days 3 and 4 of the estrous cycle (day 0 = ovulation) and it reaches maximal diameter between days 12 and 16 of the cycle.<sup>28</sup> Ultrasonic detection of corpora lutea may be more accurate than detection by palpation, but this is dependent on the experience of the individual performing rectal palpation.<sup>14,30,32,44</sup> Detection of a CL with ultrasound is based on differences in echogenicity between the stroma and the luteal tissue, whereas CL detection by palpation is based on the presence of a crown protruding from the ovary, a discernible, defined structure within the ovary and/or total ovarian size.<sup>33</sup>

The ability to discern CL from the surrounding stroma depends on the quality of the ultrasound equipment and the skill of the ultrasound technician. Occasionally it can be difficult to differentiate the CL from the stroma due to the size of the CL and the area of the ovary occupied by the corpus luteum. Usually the stroma can be differentiated from the CL by the presence of numerous small follicles dispersed throughout the stroma.<sup>13</sup> Ultrasound machines with expanded gray scale capabilities enhance the ability to differentiate ovarian structures due to subtle differences in echogenicity.

Embryo transfer practitioners often reject recipients presented for transfer based on the absence of palpable luteal tissue or the presence of a small, irregular, fluid-filled or soft CL. Ultrasonography may provide a better method of evaluating corpora lutea in embryo transfer recipients (Beal, unpublished data). When embryos were transferred to recipients that had acceptable luteal tissue based on ultrasonography (solid CL >13mm or a fluid-filled CL with at least 3 mm of luteal tissue uniformly surrounding the central cavity), but that had been rejected based on rectal palpation of the CL just prior to transfer, the pregnancy rate of the recipients was similar to that of recipients whose CL's had been classified as "satisfactory" or "excellent" by rectal palpation. In fact, 79% (96/121) of the recipients that would have been rejected because of an unsatisfactory CL based on palpation became pregnant. It is recommended that, if there is a question about the suitability of a CL after performing rectal palpation, the ovary can be scanned with ultrasound and a decision made on whether to transfer to that recipient.

Follicular cysts are non-echogenic structures with a thin wall (2mm or less) and are 25mm or larger. In addition to their large size and absence of luteal tissue, follicular cysts may be distinguished by coincident estrous behavior and low plasma progesterone concentrations.<sup>19,21,40,43</sup> Changes in the appearance of a follicular cyst following treatment with gonadotropin releasing hormone (GnRH) have been monitored using ultrasonography.<sup>19,49</sup> During the 14 days after treatment with GnRH, the wall of the follicular cyst increased in width from 2 to 6 mm due to the appearance of luteal tissue. Ultrasound-guided injection of human chorionic gonadotropin (hCG) directly into follicular structures has also been described.<sup>27</sup>



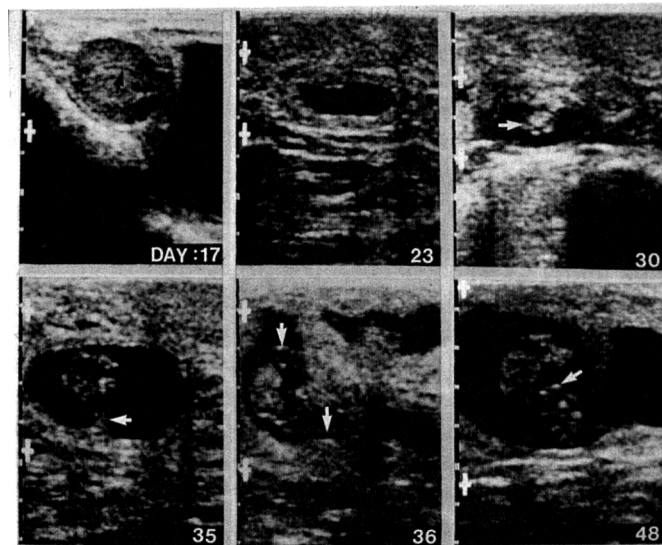
**Figure 3.** Gross appearance (left) and ultrasound image (right) of a 45-mm luteal cyst. Note: luteal tissue is scarce and not evenly distributed around the fluid-filled central cavity.

### Ultrasound Examination of the Uterus

Ultrasonic appearance of the bovine uterus is dependent on the stage of the estrous cycle. Variation in the appearance of the uterus involves changes in endometrial thickness, vascularity and the presence of intraluminal fluid.<sup>3,36</sup> The changes in endometrial echotexture are attributed to development of edema that increases in uteri of non-bred heifers beginning around day 16 and continues until day 20 of the estrous cycle.<sup>15</sup> During estrus the endometrium is noticeably echogenic, the endometrial/myometrial border is obvious and small accumulations of fluid occur throughout the uterine lumen.<sup>15</sup> The echogenicity and “puffy” appearance of the uterine endometrium decreases by 4 or 5 days after ovulation.<sup>3,36</sup> The uterine horns are extended during and shortly after estrus, but become highly coiled under the influence of progesterone during the luteal phase.<sup>36</sup>

Real-time, B-mode ultrasonography has been reported to detect pregnancy in cattle as early as 9<sup>4</sup> or 12 days<sup>38</sup> into gestation. Other reports, however, have disputed those claims and emphasized that accuracy of ultrasound diagnosis of pregnancy on day 10 through

16 was not significantly better than a guess ( $\leq 50\%$ ). Accuracy of diagnosis improved, however, by day 18 (85%), 20 (100%) and 22 (100%) of pregnancy.<sup>25</sup>



**Figure 4.** Ultrasound images of bovine embryos and fetuses; Days 17 to 48. (Courtesy of O.J. Ginther and R.A. Pierson)

Ultrasonically, the embryo proper is defined as a distinct echogenic structure within a nonechogenic, fluid-filled vesicle. Presence and vitality of the embryo can be confirmed by the detection of a heartbeat as early as 19 to 24 days of gestation.<sup>8</sup> The embryo initially appears as a short, straight echoic line (20-22 days), later becomes C-shaped (22-30 days) and finally, by 30-32 days of gestation, assumes an L shape.<sup>8</sup>

The potential advantages of using ultrasonography for pregnancy diagnosis are that the presence of an embryo can be detected earlier than by palpation *per rectum* and that direct physical manipulation of the gravid reproductive tract is unnecessary with ultrasonography. The latter fact should reduce the risk of inducing embryonic mortality or atresia coli. An association between early, vigorous palpation *per rectum* of the amniotic vesicle (prior to day 42 of gestation) and atresia coli in calves has been observed.<sup>34</sup> Vascular insufficiency to the developing spiral colon caused by damage to the colonic blood supply during aggressive palpation of the amniotic vesicle has been suggested as a probable mechanism.<sup>6</sup> Use of ultrasonography rather than palpation *per rectum* may also improve consistency of early (<45 days) pregnancy diagnosis by reducing the variation in accuracy among practitioners.

The efficiency (speed and accuracy) of detecting early pregnancy with ultrasound is markedly increased when the embryo can be detected more easily. Although

the embryo can first be detected between days 19 and 24 of gestation, when scanning large numbers of cattle, it is most practical to scan females expected to have embryos >24 days of age.<sup>14,45</sup>

The ability to identify non-pregnant cows with ultrasonography earlier than by rectal palpation can be of economic benefit to beef and dairy producers.<sup>11,22,46</sup> Some have reported using ultrasound as early as 21 days after insemination to identify non-pregnant cows.<sup>46</sup> The accuracy of a negative diagnosis (open) reached 100% by day 28 post insemination<sup>14</sup> or by day 33 in another study.<sup>46</sup> One study reported a reduction of as many as 40 days open per pregnancy in embryo transfer recipients.<sup>22</sup>

The effect of poisonous plants on the developing fetus has also been investigated using ultrasonography. Real time ultrasound has revealed significant reduction in fetal movements in sheep following ingestion of piperidine alkaloid-containing plants.<sup>39</sup> Ultrasonic imaging provides another method of observing the effects of noxious weeds on fetal development, possibly improving our understanding of how poisonous plants may affect reproductive processes in other species of livestock.

The ultrasonographic appearance of abnormal uterine fluid can vary from anechoic fluid with floating particles (referred to as 'snowy specks') to a homogenous, flocculent exudate that can appear similar in echogenicity to the surrounding uterus. In cows with endometritis, the uterine fluid containing echogenic particles can easily be distinguished from the clear non-echogenic fluid of the periovulatory period or early pregnancy. The presence of a thickened uterine wall associated with metritis can also be identified with ultrasound. In cows diagnosed with pyometra, the fluid contains diffuse, echogenic particles within the distended uterus as well as a thickened uterine wall. The viscous fluid may resemble uterine tissue but can be distinguished by the flowing motion of the exudate within the lumen. Mucometra and hydrometra are often associated with segmental aplasia of the uterus. Here, a thin walled uterus appears to be full of echogenic particles<sup>15,40</sup> Ultrasound offers an objective method to assess treatment progress and to differentiate tissue character associated with pathology of the bovine reproductive tract<sup>20</sup>

### Determination of Fetal Viability and Age

Curran and coworkers<sup>8</sup> characterized the growth of the embryo proper from 20 through 60 days of gestation, and determined when characteristics such as the heartbeat (day 22), spinal cord (day 28), placentomes (day 35), split hooves (day 44), and ribs (day 52) first became detectable. Subjective evaluation of the development of anatomical traits can be used to age bovine

fetuses, but the most accurate estimate of gestational age is derived from actual measurements of specific features. The regressions and correlation coefficients between the development of the bovine fetus and age of gestation were obtained for at least 25 different features.<sup>23</sup> Measurements of crown rump length, head diameter and trunk diameter are the easiest predictive measurements to use for estimation of gestational age (Table 2; Figures 5 and 6). In addition, the use of these measurements in formulas to estimate age results in the least variation between the estimated and actual ages. Crown-rump distance is that measured from the tailhead to the greater curvature of the skull. It is most easily measured in embryos or fetuses presented in the frontal or sagittal view. Head and trunk measurements are recorded at their maximal diameters (braincase just caudal to the eyes, and abdomen near the umbilicus). A cross-sectional or frontal presentation is required to record head or trunk measurements. Experience has revealed that crown-rump length is best for estimating ages of embryos less than 50 days, and head or trunk diameters are more easily obtained for fetuses over 50 days old. When the date of calving was determined for one of these three measurements, the actual day of calving was predicted within an average of 4.5 days (crown-rump), 6.9 days (head) or 7.8 days (trunk).<sup>48,50</sup>

The rate of early embryonic death between days 25 and 90 was estimated at 10% ± 5% when based on uterine fluid alone, but the estimate decreased to 6% ±

**Table 2.** Formulas for determination of fetal age from ultrasonic measurements.<sup>1</sup>

Measurement	Day of Gestation =
Head diameter (cm)	(log of head dia.) x (45.23) + 37.7
Trunk diameter (cm)	(log of trunk dia.) x (37.21) + 39.7
Crown-rump (cm)	(log of crown-rump) x (16.73) + 27.5

<sup>1</sup>Formula requires natural log (ln) function available on most pocket calculators.



**Figure 5.** Ultrasound image of fetal head; diameter 2.0 cm; estimated age 69 days.

**Figure 6.** Ultrasound image of crown-rump; length 3.5 cm; estimated age 48 days.





5% when the diagnosis of pregnancy was based on visualization of an embryo.<sup>14</sup> Others have reported early embryonic mortality rates of 6%,<sup>45</sup> 8%<sup>50</sup> and 15%<sup>22,46</sup> during the first 90 days of gestation. Knowing this, it is recommended that the pregnancy status of all cows diagnosed pregnant before 35 days be reconfirmed after 60 days.

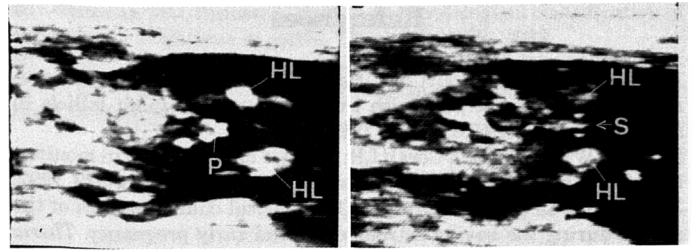
Macerated fetuses may appear as distorted images surrounded by purulent fluid characterized by anechoic background fluid containing echogenic particles. Degrading embryonic tissues within the vesicle increase the echogenicity of the amniotic fluid surrounding the embryo, which may appear distorted as well. Frequently, these changes are too subtle to be detected by palpation. Sometimes, the fetus may retain its shape but a heartbeat cannot be detected and the amniotic vesicle may appear gray due to the degenerating debris from the dead fetus while the surrounding allantois maintains its non-echogenic appearance. Mummified fetuses often appear as a poorly defined echogenic intrauterine mass but without surrounding fluid. Occasionally, the bones may be identified as dense echogenic tissues shadowing the tissue below. A thickened uterine wall may also be apparent.<sup>15</sup>

Retention of a non-viable embryo is usually accompanied by the maintenance of the corpus luteum. Cows treated with prostaglandin to induce luteolysis experience rapid expulsion of the embryonic tissue (<24hr) and return to estrus within 5 days. In a recent study, over 50% of the cows treated to expel a non-viable fetus conceived to the first AI following treatment (Beal, unpublished). Hence, detection of a non-viable embryo can be followed quickly by a return to pregnancy if the animal is treated and rebred.

### Determination of Fetal Number and Gender

The bovine genital tubercle is an embryonic structure that gives rise to the clitoris in the female and to the glans penis in the male. It originates between the rear legs of the fetus and migrates to a position just caudal to the umbilicus in the case of the male and ventral to the anus in the female. After day 50 of gestation, male and female fetuses can be differentiated by relative location of the genital tubercle (presumptive penis or clitoris) and development of genital swellings into a scrotum in the male fetus. Diagnosis of sex should be made by visualization of either male or female sex organs and should be nearly 100% accurate. Determinations made on the basis of absence or inability to identify the organs either ventral to the tailhead or caudal to the umbilicus may result in lower accuracy.

Ultrasound imaging of bovine fetuses on day 48 to 119 has been performed to determine fetal sex.<sup>2,7</sup> Here, the ultrasound transducer must be manipulated



**Figure 7.** Ultrasound images of a male fetus (68 days; frontal). Left panel shows hindlimbs (HL) and penis (P). Right panel shows scrotum (S).

within the rectum to provide a frontal, cross-sectional or sagittal image of the ventrum of the fetus. The umbilicus and tail serve as excellent landmarks when determining the location of the genital tubercle or the presence or absence of the scrotum. The accuracy of fetal sexing can be optimized by proper timing in relation to fetal age. Sex determination prior to day 60 is more difficult because the relative migration of the tubercle is not complete. Conversely, when scanning after day 85, two situations occur which may reduce accuracy. First, as the fetus gets larger it becomes more difficult to move the transducer relative to the fetus to attain the desired image. Second, the gravid uterus is more likely to have descended over the pelvic brim which makes sexing impossible without retraction of the gravid horn. Concurrent retraction of the horn and handling of the transducer is difficult and manipulation of the horn increases the risk to the fetus. Because of these factors, the best window for sexing fetuses is between 60 and 85 days of gestation.

Ultrasound can be accurate in distinguishing cows carrying one or more fetuses.<sup>9,12</sup> One study reported a slight increase in accuracy by evaluating fetuses at 51 versus 43 days of gestation<sup>9</sup> while a second study reported improved accuracy after 45 days of gestation.<sup>12</sup> They also concluded that the uterine size after day 80 limited thorough examination of the uterus and fetal counts at that stage were not reliable.

### Conclusion

Ultrasound has the potential to improve diagnostic methods used in traditional veterinary practice. The greatest application is to improve and extend the capabilities of rectal palpation. To justify the cost of ultrasound equipment, the use of ultrasound must be incorporated into other areas of the practice. Modern equipment is versatile and the same equipment can be used for equine, bovine and small animal diagnostics. The areas discussed in this paper are the most likely, but certainly not the only applications of ultrasound for bovine reproductive management.

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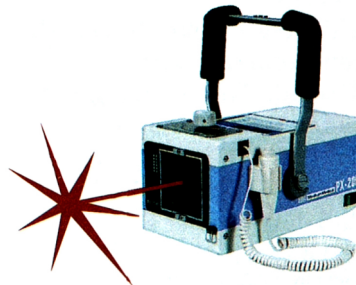
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