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Bovine Fetal Sexing Using Ultrasound

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Introduction

The economics of food animal production is the driving force behind advanced reproductive technologies in the cattle industry. For the past 15 years, the use of ultrasound has proven to be a valuable tool for cattle breeders to assess carcass characteristics and to provide valuable reproductive information beyond the scope of rectal palpation. There are many reproductive scenarios that ultrasound addresses; early pregnancy diagnosis as early as day 21 (Pierson et al., 1984), normal vs. cystic ovarian disease (Pierson, 1984), cycling vs. anestrous females, multiple pregnancies (Stroud, 1991), early embryonic death (Fissore et al., 1986), live vs. dead fetus, response to superovulation (Guibault et al., 1991), oocyte aspiration for IVF (Callensen et al., 1987), endometritis (Fissore, 1988), and fetal sexing. Knowledge of the sex of a fetus, male or female, by days 60 – 90 of gestation provides extremely valuable management information for breeders.

Fetal sexing by ultrasound was first reported in the early 1990's (Curran et al., 1991). Since then, tens of thousands of first trimester pregnancies have been diagnosed by skilled ultrasonographers. The accuracy of the procedure is determined primarily by the skill and experience level of the technician, quality of the ultrasound unit, and the ambient conditions during the examinations, but experienced personnel should be at least 95% accurate in diagnosing fetal sex. Since 1993 the author has performed more than 12,000 fetal sexing procedures with less than five reported missed diagnoses.

Physics of Ultrasound

The physics of real-time ultrasonography have been described in elaborate detail by previous investigators (Pierson et al., 1988), but for the purpose of this article, a brief overview should suffice. A transducer, or probe, has an array of crystals that, when electrically stimulated, produce high-frequency sound waves in a linear, convex linear, or sector (pie-shaped) direction. For bovine reproductive applications, a linear-array transducer is used transrectally in order to facilitate proximity (one to three inches) to the target object. A highly resolved and focused image is thus produced. A linear transducer transmits ultrahigh frequency (inaudible) sound waves along a three- to four-inch axis. The width of the ultrasound waves is approximately one millimeter; therefore, any image projected on the monitor would be comparable to viewing the same structure at necropsy that is opened by a knife in either cross, longitudinal, or oblique sections.

The transmitted sound waves travel through body tissue in a direction determined by the angle of the transducer until they reach a dense tissue reflector. Some of the sound waves are absorbed (fluid) and some are reflected (various tissues and bone) and return to receiving crystals in the transducer. The force of the returned waves compresses and expands the crystals which, in turn, produce a voltage that is amplified and converted into lifelike images on a high-resolution monitor.

Tissues have different densities that reflect sound at various amplitudes (strengths). For example, the echo produced from amniotic fluid would be weak or anechoic (black on the monitor), whereas the echo from fetal bone, a dense tissue, would be strong or highly echogenic (almost white on the monitor). Significant reproductive tissues of the bovine uterus and ovary (follicular and luteal tissue) as well as various fetal organs have different densities and therefore reflect sound at various amplitudes. These densities are depicted as various shades of gray on the monitor. Most modern, linear ultrasound units produce at least 128 shades of gray that result in high-resolution images of clinically important tissues. The gray-scale image is refreshed with current data at the rate of 30 frames-per-second thus creating a *real-time* or moving image. Figuratively, a real-time ultrasonogram is similar to a moving x-ray.

Applications of fetal sexing

The management applications of fetal sexing by ultrasound are numerous. Prior knowledge of the sex of a fetus can influence the sale value of bred heifers or cows especially in the purebred industry. Also, grouping bred heifers by sex of fetus can be advantageous for calving since the incidence of dystocias are significantly higher with male calves than female. In the dairy industry, sexing the fetus of a marginally efficient older cow can determine whether or not she should be culled if she carries a bull or heifer calf inside. In the case of twins, ultrasound fetal sexing can distinguish between same sex twins and freemartins. Additionally, embryo transfer recipients can be sexed to determine if an adequate number of a desired sex has been achieved from a particular flush or group of transfers. For example if a breeder has sold a flush with a guarantee of two heifer calves, and only one recipient is diagnosed as having a female fetus, the donor should be flushed again in an attempt to satisfy the terms of the sale. There are numerous other scenarios where knowing the sex of the fetus can be advantageous to the owner or buyer of a particular female.

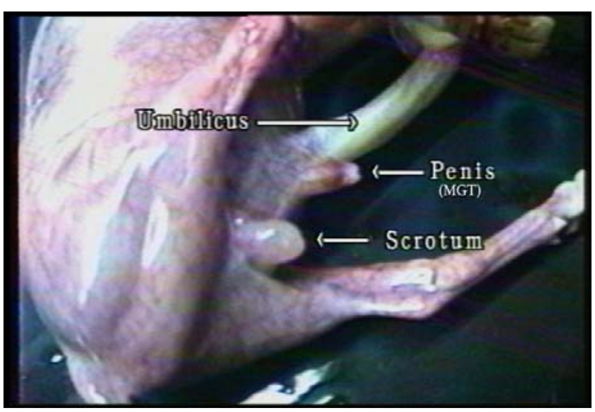


Figure 1

Figure 2

Fetal anatomy

At approximately day 60 of gestation, male and female genital tubercles can be visualized on a high-resolution ultrasound monitor. The fetal sex organs are composed of dense, highly echogenic tissue similar to skeletal structures and therefore are depicted as bright or white structures on the monitor. Male and female genital tubercles appear bilobed on the monitor; each lobe is in the shape of an oval, which aids in differentiation from surrounding structures[6]. The male genital tubercle is found just caudal to the umbilicus (Figure 1), whereas the female genital tubercle is located under the tail (Figure 2).

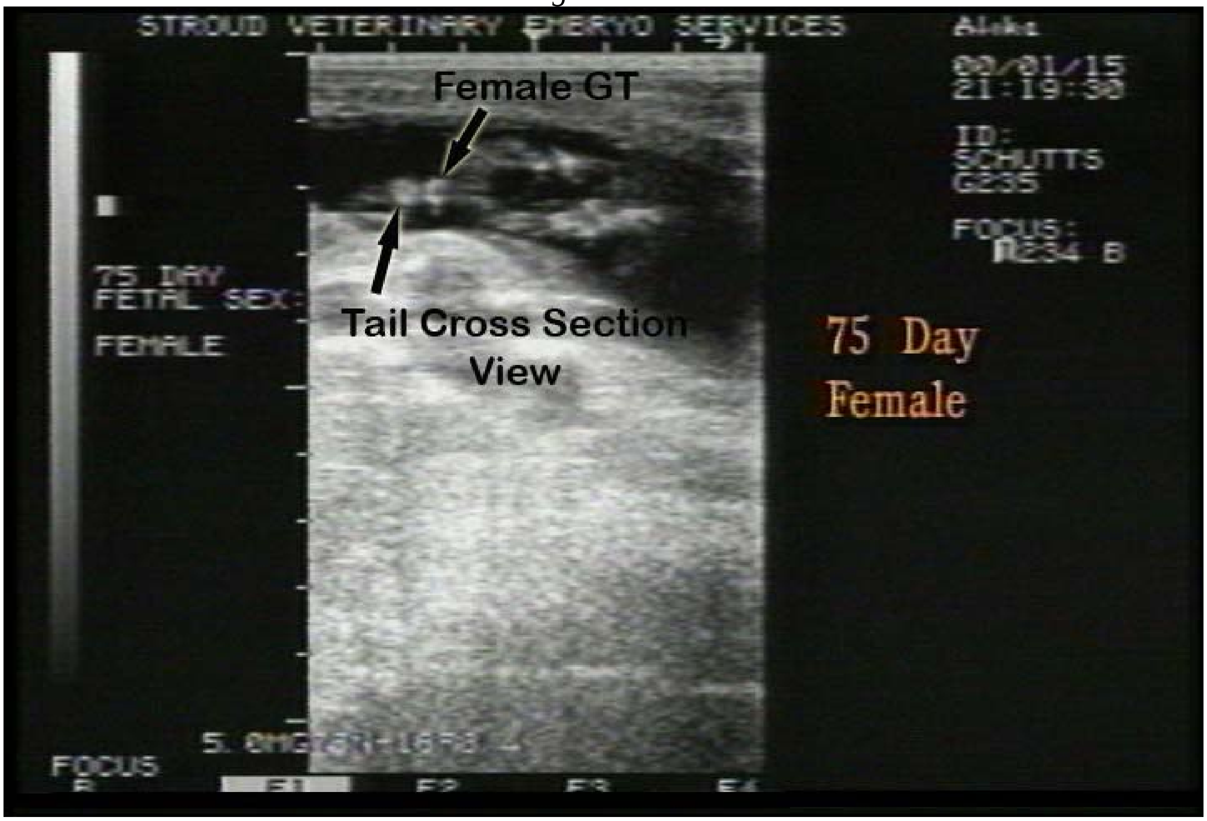
Fetal sex examination

A systematic approach should be taken by the ultrasonographer when performing fetal sexing. There are three very important anatomic references on a fetal sonogram that are critical in achieving proper orientation of the fetus: (1) the head, (2) the beating heart, and (3) the umbilicus (Stroud, 1994). These structures are relatively easy to recognize on an ultrasound monitor. It is sometimes difficult to differentiate the front legs from the rear legs; therefore, these structures have been excluded from the list of anatomic references. Once the fetus has been located on the monitor, the three anatomic references should be systematically examined to ensure cranial-to- caudal orientation.

The following three views can be used to observe a fetus during an ultrasonographic examination: a lateral view (seldom seen), a frontal view (routinely seen and easiest for orientation), and a cross-sectional view (the most often presented). Angled or oblique variations of these views are often presented during routine ultrasound exams, but, for teaching purposes, all three views are discussed in principle.

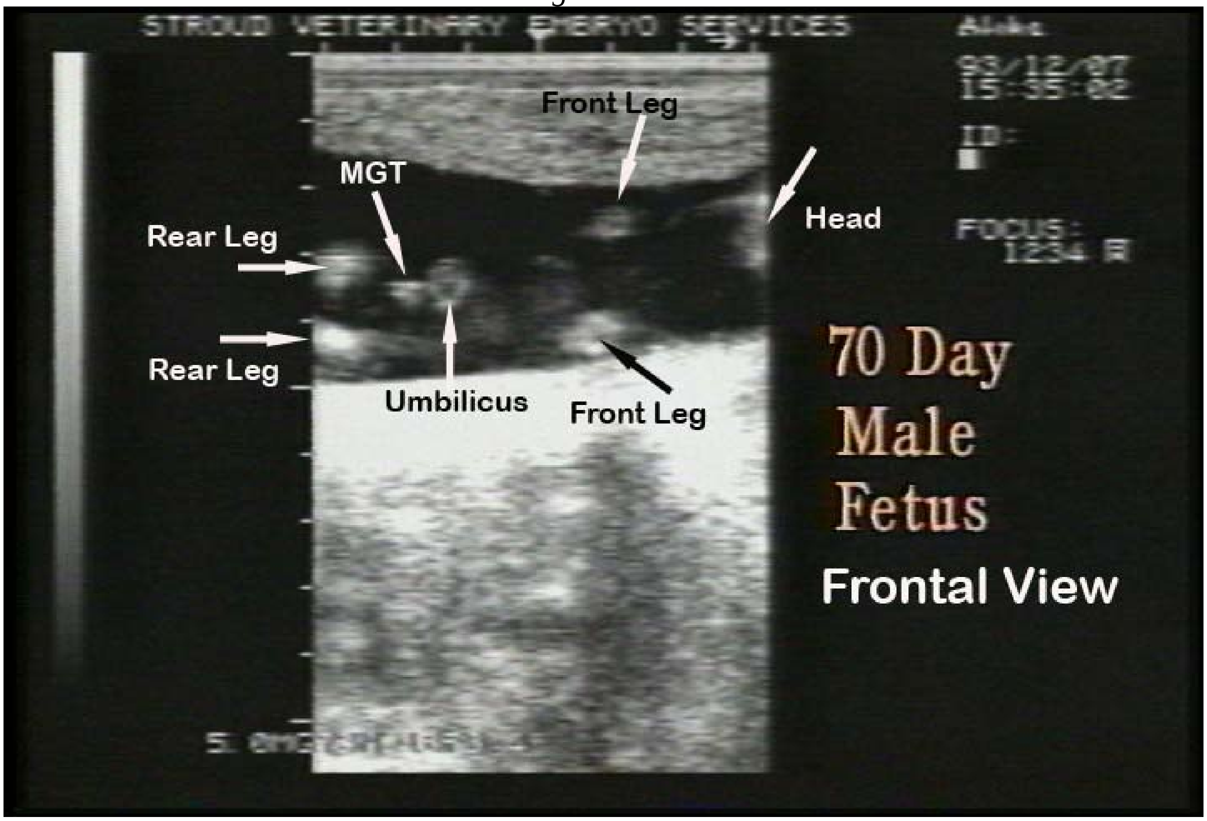
During a cross-sectional examination of the fetus, the transducer is placed over the cranium and moved distally through the thorax to review the beating heart; no heartbeat indicates a dead fetus. The transducer is moved further distally to where the umbilicus attaches to the abdomen. At this time, the transducer should be moved slowly back and forth to diagnose the presence or absence of a male genital tubercle. In males, the genital tubercle is immediately caudal to the umbilicus, appears very bright or highly echogenic on the monitor, and is usually bilobed.

Figure 3



If a male genital tubercle is detected, the examination is complete. If a male genital tubercle is not observed, the transducer must be moved distally to the perineal area to detect the presence of a female genital tubercle. The perineal area is the most difficult region of the fetus to focus; therefore, patience is required. The ultrasonographer should move the transducer slowly and must establish the difference between a cross-sectional view of the tail and the female genital tubercle (Figure 3). The female genital tubercle is generally bilobed, whereas the tail is a monolobed structure. Frequently, the tail and female genital tubercle are seen simultaneously and the ultrasonographer should definitively distinguish one structure from the other.

Figure 4



When the fetus is in a frontal position, the head, thorax, abdomen, and inguinal area can be viewed. The transducer should be manipulated so that the umbilical attachment to the abdomen comes into focus. In males, immediately caudal to the umbilicus is the hyperechogenic male genital tubercle (Figure 4). The frontal view is excellent for diagnosing gender because the perineal area can also be viewed; however, some finesse by the technician is required. The female genital tubercle is sometimes superimposed over the tail. If the transducer is titled either to the left or right, creating a slightly oblique angle, the two structures can be effectively separated optically.

Lateral-view orientation is presented occasionally. From the author's experience, the female genital tubercle is somewhat difficult to visualize using this position. The male genital tubercle at 60 to 100 days and often the entire sheath/prepuce/penis complex of a 90-day pregnancy examination is easily seen on a lateral-view ultrasonogram.

Common mistakes

Before a definitive diagnosis of fetal sex is made, it is imperative that the respective male or female genital tubercle is seen clearly and distinctly by the ultrasonographer. Diagnosing male or female based on the absence of either genital tubercle is ill advised. For example, it is usually faster to diagnose a male simply due to the fact that the penis happens to be located near the attachment of the umbilicus to the abdomen. Since the umbilicus has such an optical presence in an ultrasound exam, it's easy to find, and traceable to the abdomen where the male genital tubercle resides.

However, during some examinations of male fetuses, when the transducer is placed at certain angles, ultrasound waves can become scattered or reflected creating an unresolved and undiagnosable image of the male genital tubercle. So, just because a male genital tubercle is not observed at first glance doesn't mean that the fetus is a female. If a male genital tubercle is not observed, the ultrasonographer must move to the rear of the fetus and see a female genital tubercle before making a final decision.

Conversely, a female fetus can be misdiagnosed as male when the tail is tucked between the hindlegs.[7] The tip of the tail can actually approach the area close to where the umbilicus attaches to the abdomen and create a hyperechogenic structure similar to a male tubercle on a cross-sectional view. Ultrasonographers must be patient and decisive in order to avoid misdiagnosis. With experience, making an accurate diagnosis should not be a problem.

Figure 5

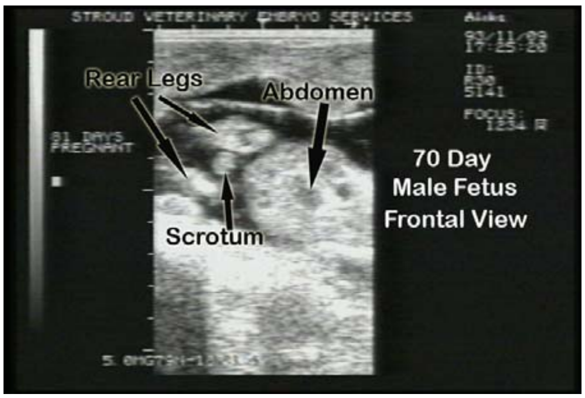
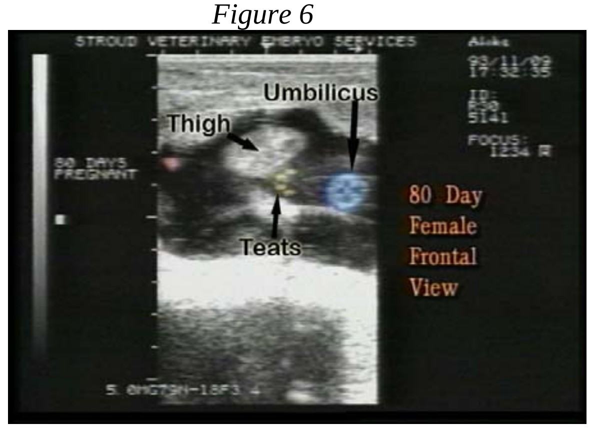


Figure 6



At approximately 75 to 90 days of gestation, fetal sexing is enhanced by secondary reproductive anatomic structures. In males, the scrotum has developed and can easily be seen on a frontal view between the rear legs (Figure 5). In females, the teats are very distinct in the frontal (Figure 6) and cranial-caudal views. Ultrasonographers must be careful when scanning 90 to 120 day male fetuses because some will display rudimentary teats. Also, inexperienced ultrasonographers sometimes see hyperechogenic bits of tissue that can be misconstrued as teats on a female or a scrotum on a male. So, diagnosing sex based on the presence or absence of secondary reproductive structures is not advised. However, once an ultrasound technician becomes confident with ultrasound anatomy, the scrotum and teats are helpful adjuncts to the genital tubercles when diagnosing sex.

Learning curve

Ultrasonographers must (1) have a thorough understanding of ultrasonographic fetal anatomy and (2) develop the skills necessary to produce fetal images that are positioned and focused well enough to accurately diagnose sex. As soon as these criteria are met, ultrasonographers will become proficient in determining fetal sex. A considerable amount of practice is needed in order to achieve a professional level of expertise in making a consistent and accurate diagnosis. Reaching that level can be quite frustrating, but, with patience, it can be done in a reasonable time frame. The author recommends a two phase learning curve.

Phase one involves learning to accurately read images of both male and female fetuses at various stages between 60 and 90 days and at different angles, i.e., frontal, cross sectional, and obliques. Studying quality still images captured from a sonogram is a good way to begin. Structures such as the umbilicus, head, heart, and fetal sex buds should become recognizable on still images before moving to real time ultrasound exams. Once stills have become mastered, the student should have the confidence to move to videotaped real time exams. Studying edited videotapes with labeled structures transitioning into unedited real time exams can save dozens of hours of frustration for upstart ultrasonographers.

Phase two is simple in principle, but very difficult for most students – producing a quality image with arm in cow. Without having conquered phase one, phase two can be daunting. Assuming phase one has been completed, producing quality images will likely take at least 200 or more exams. The first 50 or so often frustrates many aspiring veterinarians to the point of quitting. Patience and stubbornness are required. The author recommends beginning with five or so exams at a time then progressing to more as confidence grows. Combining both phases culminates in a practitioner being able to accurately diagnose sex.

Selecting an ultrasound unit

A dozen or more companies are currently marketing veterinary ultrasound units in the United States. Major considerations in making a selection are resolution quality, price, serviceability, portability, availability of new as well as loaner units, and the willingness on the part of the salesperson to educate the buyer before and after a sale. The cost of veterinary ultrasound units ranges between $3000 and $20,000, depending on the resolving capabilities, number of transducers, and other technical features.

For most clinical bovine reproductive applications a 5-MHz linear array transducer to be the most versatile and effective. That unit performs adequately on early pregnancy examinations; fetal sexing; pathologic ovaries; and, in general, most all reproductive uses. A 7.5-MHz linear transducer may be more practical if the ultrasonographer intends to do research on follicular dynamics. For transvaginal oocyte recoveries for in vitro embryo production a convex linear transducer gives the technician much more flexibility in gaining access to the hard-to-reach follicles as compared with a linear transducer.

If at all possible, a buyer should sample any potential ultrasound unit and ask for a list of buyers to get feedback before purchase. Most major veterinary conventions have representatives on the trade floor that are more than happy to show their product; however, live cows are recommended as the test host. If portability is a major concern, the buyer should definitely consider the size, weight, stability, and the intended usage for the unit. For example, if fetal sexing is to be done heavily in an ambulatory practice, resolution and portability are major concerns and the unit should be tested under those conditions before purchase.

Intangibles

Some intangible benefits arise from using ultrasonography in practice. Ultrasonographers inevitably become more proficient in rectal palpation. The difference between a luteal cyst and a normal fluid-filled follicle is easily discernible by real-time ultrasonography but is very subtle by rectal palpation. After having viewed several hundred of each via an ultrasonographic examination, diagnosis by palpation becomes easier. The same holds true for early pregnancy testing. The art of palpation takes literally thousands of cows and years of practice to become proficient. With the help of real-time ultrasonography, an individual inexperienced in rectal palpation could learn skills much more quickly while simultaneously providing a more accurate diagnosis to clients.

Conclusion

Fetal sexing by ultrasound has seen limited use over the last decade due to the steep learning curve necessary to become proficient. However, video training along with well organized short courses with wet labs over the last few years are turning out some well qualified ultrasonographers. Once clients have had bred females accurately sexed they soon demand the service routinely. Having the knowledge of sex before birth is very valuable information. When combining fetal sexing with the other benefits of ultrasound, breeders of valuable purebred livestock begin to rely on the technology.

The bottom line is that ultrasonography in a bovine practice can be profitable to both veterinarians and their clients. Veterinarians must understand that the learning curve is time consuming and sometimes frustrating. The initial investment in a high-quality ultrasound unit also warrants considerable deliberation—ultrasound units are expensive. An extremely busy practitioner may not have the time to learn how to use the unit, which would make its purchase ill-advised; however, if bovine veterinarians want to improve their image, enhance their diagnostic skills, and become leaders in a relatively new discipline of clinical veterinary medicine, ultrasonography may be the tool to achieve these goals.

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