The Use of Ultrasound in Cow/Calf Applications

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Abstract

Transrectal guided real-time ultrasound has been used extensively to diagnose reproductive scenarios in the bovine female for almost 20 years. Uterine diagnoses include early pregnancy, multiple pregnancies, sex of the fetus, exudates in the uterine lumen, early embryonic death, stage of the estrous cycle (estrus vs. diestrous), and other abnormal conditions. Ovarian diagnoses include follicular dynamics, stage of estrous cycle (estrus vs. diestrous), presence or absence of corpora lutea, cystic ovarian disease, response to super stimulation of donors at estrus and diestrous, ovarian tumors, polycystic ovaries, and anestrous vs. cycling females. In addition, ultrasound has been used as an instrument for collecting oocytes for *in vitro* fertilization. This paper reviews the procedures, discusses the efficacy of the diagnoses of the various conditions, and reflects on the scenarios most commonly encountered in bovine practice. The learning curve for using ultrasound will also be thoroughly reviewed.

Résumé

L'échographie transrectale dirigée en temps réel a été utilisée fréquemment pour diagnostiquer les problèmes reproducteurs chez les bovins femelles depuis près de 20 ans. Les diagnostics utérins incluent la gestation précoce, les gestations multiples, le sexe des fœtus, la présence d'exsudats dans la lumière utérine, la mortalité précoce des embryons, le stade du cycle œstrale (œstrus versus dioestrus) et d'autres conditions anormales. Les diagnostics ovariens incluent la dynamique folliculaire, le stade du cycle œstral (œstrus versus dioestrus), la présence ou absence de corps jaune, la maladie kystique ovarienne, la réaction à la super stimulation des donneuses à l'œstrus et'au dioestrus, les tumeurs ovariennes, les ovaires polykystiques, et l'anovulation versus la cyclicité chez les femelles. De plus, les ultrasons ont été utilisés dans le prélèvement des ovules pour la fertilisation *in vitro*. Cet article fait la revue des procédures, offre une discussion sur l'efficacité des diagnostics pour différentes conditions et présente les scénarios les plus souvent rencontrés en pratique bovine. La courbe d'apprentissage pour l'utilisation des ultrasons est aussi discutée en détail.

Introduction

The physics of real-time ultrasonography have been described in elaborate detail by previous investigators[7], 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 cut 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 is reflected and returns 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 life-like 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 bovine intrapelvic reproductive tissue of the 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 128 to 256 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.[2] Figuratively, a *real-time* ultrasonogram is similar to a moving x-ray that can be used to diagnose reproductively important images in the cow or heifer.

Early Pregnancy Diagnosis

The ability to definitively diagnose pregnancy on or before the 27th day of gestation (day 0 = standing estrus), is important, especially in intensive breeding programs.[3] The ability to diagnose an exposed cow as open (not pregnant) for immediate resynchronization and rebreeding is perhaps even more important. This can have a profound effect in decreasing the total number of open days in intensive embryo transfer and artificial insemination programs. Fortunately for the budding ultrasonographer, early pregnancy diagnosis is very easy to learn, assuming the student has some palpation skills. If the transducer can be placed directly over the uterine horns then a diagnosis of pregnancy is easy and extremely fast, even for pregnancies as early as 24 to 25 days. Both uterine horns must be examined thoroughly to see pregnancy fluids that early. The observation of a fetus is not terribly important until about day 30 or more. Diligently searching for a fetus with a heartbeat earlier than day 30 can be time consuming and a potential threat to the conceptus.

An inexperienced ultrasonographer must be able to delineate between urine in the bladder and fetal fluids in the lumen of the uterus. The edge of the bladder wall typically has a fern-like appearance as opposed to the edge of the lumen of a gravid uterus which is smoother. Also, urine tends to have hyperechogenic crystals which show up as specs floating in the non-echogenic areas. Palpation experience helps the ultrasonographer determine that the transducer is directly over the uterus and not the bladder.

On an ultrasonogram, the lumen of a non-gravid bovine uterus should not normally contain nonechogenic (black) areas. Exceptions include cows in estrus,[8] anestrous cows with a very flaccid uterus, or cows with a pathologic condition.[4] The nonechogenic line frequently observed in cows in estrus is usually visible along the entire length of the lumen of the uterus. This black line represents the secretory fluids of the uterus that are in part responsible for transportation of sperm. Normal estrus fluid sometimes pools in the greater curvature of the uterus, giving the false impression of early pregnancy fluids. An examination of the ovaries during estrus should reveal a mature follicle (> 10 mm in diameter) on one ovary and no luteal tissue on either ovary. The myometrium of a cow in estrus is toned by palpation and appears thickened on the ultrasound monitor as compared with that of a cow in diestrous or early pregnancy. A thorough reproductive history of the cow is helpful when making diagnoses. Inexperienced ultrasonographers are recommended to wait until day 30 to diagnose a female as open. By day 30, a large amount of fetal fluid can be detected and the fetal heart beats visibly.[5] A corpus luteum on the ipsilateral ovary of pregnancy helps to confirm early pregnancy if ultrasonographers are in doubt.

The gravid uterine horn in early stages of pregnancy should be examined in longitudinal and cross-sectional planes. In some cows, the uterine lumen tends to be somewhat flattened instead of round or oval. This can be confounded by the ultrasonographer exerting downward pressure on the uterus during the exam. In flat lumens, a cross-sectional examination may not readily indicate pregnancy whereas a longitudinal examination would easily do so. Previous investigators recommend little if any manipulation of the reproductive tract for an ultrasonographic examination[9]; however, in some cows, prior manual retraction of the uterus per rectal palpation is very helpful (author's opinion). An ovary or uterine horn is often tucked under the broad ligament, and digital manipulation of the organ is required in order to gain access. This allows placement of the transducer over the surface of the organ and provides a thorough examination. All of our in-house recipients and artificially inseminated cows are tested for pregnancy at day 27 or 28 via ultrasonographic examination. Routine manipulation of uterine horns (while the transducer is concurrently held) is done to ensure a thorough examination before a cow is diagnosed as open and re-synchronized.

Multiple Pregnancies

Diagnosis of twins by ultrasound is somewhat of a paradox. If there is one fetus how does one prove there isn't another? When does the ultrasonographer give up on proving a negative? The point is that diagnosing twins can be difficult. The recommendation is don't be too aggressive looking for something that may not exist. However, there are certain stages of gestation when twins are relatively easy to find. The easiest time is between days 35 and 70. The secret to diagnosing twins is simply to do a thorough exam of each uterine horn once pregnancy has been diagnosed. Seeing both fetuses simultaneously makes the diagnosis easy, but that sometimes takes some manipulation of the gravid uterus. If both fetuses are not visible simultaneously the ultrasonographer must be very careful that there are actually two separate and distinct fetuses. Manipulating the transducer can often cause the ultrasonographer to see the same fetus at different angles, thus creating the illusion of twins. One tip in searching for twins is to look for two CLs on the ovaries. That does not work in the case of monozygotic twins. Failing to diagnose twins can lead to dystocia, so a thorough exam is necessary.

Pathologic Fluids in the Uterus

Uterine pathology (endometritis or pyometra) is

often associated with fluids or exudate seen in the lumen during an ultrasound exam. As mentioned previously, non-echogenic fluids can indicate pregnancy or a female in the estrus phase of the cycle. To differentiate between pregnancy and pathology the ultrasonographer should look for a fetus or other indicators of pregnancy such as the amnion, umbilicus, or placentomes in later stage pregnancies. Often pathologic non-echogenic fluids are associated with advanced thinning of the uterine myometrium (by palpation), and during ultrasound examination have no fetal membranes. Purulent exudate usually has hyperechogenic intraluminal contents. The exudate is sometimes difficult to see until it moves back and forth in the lumen during manipulation. On occasion is appears glitter-like as it moves in the lumen. Such cows should be therapeutically flushed to remove the exudate.

Early Embryonic Death

For the sake of this paper the period of early embryonic death (EED) is defined as that part of the first trimester of pregnancy during which ultrasound can detect pregnancy (25 to 95 days post breeding). The reported incidence of EED varies depending on such factors as nutrition, enzootic disease, and other causes.[1,11-12] More recently embryos produced by *in vitro* methods and by somatic cell nuclear transfer (SCNT) have proved to show dramatic increases in EED (authors observation). In the initial stages of early embryonic death, palpation is inadequate to make an accurate diagnosis[10]. Such cows seem to be pregnant on rectal palpation based on ballottement of fetal fluids. With the use of real-time ultrasonography, however, an early diagnosis is possible[6]. An examination of the fetus as early as day 30 reveals the cessation of a heartbeat during cases of EED. Separation of fetal membranes from the uterus as well as echogenic specks of dead tissue floating in the fetal fluids are also detectable via ultrasound in cases of EED. In advanced stages of EED, the fetal image is distorted, shows no limb movement (except when manipulating the uterus digitally), and is sometimes invisible even though the volume of fetal fluids appears normal.

A diagnosis of embryonic death is often made during fetal sexing or when a client prefers that an ultrasonographic examination (instead of palpation) be performed to pregnancy test a cow or heifer consigned to a sale. A diagnosis of embryonic death made via an ultrasonographic examination saves not only dollars but potential ill feelings among the buyer, seller, and a veterinarian who may have misdiagnosed a pregnancy by palpation alone. In our embryo transfer facility, we routinely examine all pregnant recipients at day 90 via ultrasonographic examination before releasing them to a client, preferably with him watching. Between day 27 (initial examination of pregnancy) and day 90 of pregnancy, a 6% embryonic death loss is evident with routine embryo transfer.

Stage of the Estrous Cycle

With the application of advancing reproductive technologies such as timed AI, embryo transfer, IVF and cloning, veterinarians are often called upon to diagnose the stage of the estrous cycle of females in certain situations. For example, is a female in estrus to be inseminated or is she diestrous with a corpus luteum? Palpation is about 80% efficient in palpating corpora lutea (author's opinion). Ultrasound, on the other hand, takes the diagnosis to at least a 95% level of certainty. Corpora lutea can not be accurately aged by size or shape, but there presence gives the practitioner a lot of information about a female.

A female in or very near estrus will have a large follicle (>10mm in diameter), will have a thickened myometrium, and will typically have estral fluids in the lumen of the uterus. There will also be no observable corpus luteum (CL) on either ovary. Palpation of a corpus albicans or a regressing corpus luteum will sometimes fool a practitioner into diagnosing a diestrous state. If the cow is in estrus ultrasound will show the lack of luteal tissue even though palpation is false positive for a corpus luteum.

It's also important to determine if a female is prepuberal or anestrous before spending a lot of money trying to synchronize her for breeding or as a recipient female. Non-cycling females typically have a very flaccid uterus and no luteal activity on either ovary. Again, palpation is about 80% effective in making that diagnosis, whereas ultrasound bumps the reliability to about 95%.

In embryo transfer operations, poor heat detection of recipient herds is one of the most important factors associated with low conception rates. Some clients/managers don't heat detect their recipients at all. Instead, they rely solely on the practitioner's ability to palpate a corpus luteum at transfer time. Since embryos are recovered from donor females seven days post-estrus, the recipient should be 6, 7, or 8 days post-estrus for the best chance of getting a pregnancy post transfer. That puts the recipient in a diestrous state with a CL. Even ultrasound has its limitations in these situations. The presence or absence of a CL can be diagnosed, but the age of the CL is very difficult to determine even by ultrasound.

Response to Superovulation

One of the most responsible ways to utilize an extremely rare or valuable straw of frozen semen is by

superovulation and embryo transfer. Ultrasound becomes a very valuable tool prior to thawing the semen and inseminating the donor female. Scanning the ovaries pre-thaw can determine if the female has responded to the super stimulation treatment. Multiple follicles ≥10 mm in diameter is a good indication that the female has stimulated and has ovulatory size follicles. Their presence does not, however, insure that the follicles will ovulate and produce a viable embryo. The presence of only one or a few follicles does indicate that the donor has not responded to the super stimulatory treatment. In such instances, an alternate sire should be considered in order to preserve the rare or precious semen for a better day.

Ultrasound can also be used on embryo collection day. What may appear to be a good superovulatory response by palpation may prove to be unovulated follicles instead of CLs. The author has been humiliated many times by counting CLs by palpation only to be corrected by ultrasound post embryo collection. Those CLs will often show up as non-echogenic follicles that have failed to ovulate and lutenize. To be complete with this topic, and to ad confusion, rarely what appear to be unovulated follicles via ultrasound will ovulate and produce viable healthy embryos. Apparently, some follicles can ovulate and then fail to form luteal tissue while producing a healthy oocyte.

Learning Curve

A veterinarian must determine whether investment in a unit and learning its use on a professional level is practical. To become proficient in diagnosing fetal sex, early pregnancy, and normal and abnormal ovarian structures largely depends on the palpation experience level of the practitioner. The more palpation experience a veterinarian has, the faster he or she will learn to manipulate the transducer to get a focused, diagnosable image on the monitor. I have personally trained veterinarians from foreign countries who have only limited training in palpation and they have learned the technique very quickly.

There are three major obstacles to overcome when learning how to use an ultrasound unit. First, a thorough knowledge of what specific objects look like on the ultrasound monitor is imperative (e.g., normal and abnormal ovaries and female and male fetuses). Besides commercial video tutorials,[a,b] newcomers must also learn from attending wet labs or by first-hand experience. This can take a considerable amount of time and a tremendous number of cows with adequate breeding records to support them.

Secondly, beginning ultrasonographers must also be able to produce a focused and diagnosable image on the monitor. There is more to ultrasonography than placing the transducer in the rectum of a cow and aiming it in the general direction of the fetus or ovaries. Achieving quality images comes from experience on the part of the ultrasonographer. The structure (e.g., ovary or fetus) is generally in better focus when it is only two to five centimeters away from the transducer interface. This requires some manipulative skills on the part of the technician, and an understanding of the anatomy in question. A valuable tip to the student is to move the transducer slowly in the rectum when approaching the area of interest. Many inexperienced ultrasonographers tend to move the transducer too quickly, thus bypassing critical landmarks that are beneficial for orientation.

Thirdly, beginning ultrasonographers must learn how to operate the ultrasound unit to its maximum benefit. Some ultrasound units have simple control panels whereas others have rather sophisticated ones. Again, the salesperson in charge should spend time with the buyer, showing how to fine-tune the system under different conditions to obtain the maximum resolution from the unit.

To make an accurate diagnosis via an ultrasonographic examination, ambient lighting conditions should be controlled. A dark room is ideal for viewing the monitor and helps the human eye recognize as many shades of gray as possible. An ultrasonographic examination done in a well-lighted area is similar to watching television in the yard on a sunny day—the picture is very difficult to see. If there is no option but to examine cows in lighted conditions, some type of hood must be draped over the monitor to facilitate effective gray-shade delineation.

Conclusion

In this age of information and advancing technology, cattle producers are continuously seeking new ways to cut costs, quantitatively measure their products, be innovative in marketing, and, in general, be profitable. Producers look to veterinarians for advice on new medical and nutritional products and services to obtain a professional opinion on efficacy and how products and services may or may not fit profitably into their operation. Veterinarians should stay abreast of this ever-changing profession and make sensible decisions concerning their clients' long-term interest. In my opinion, bovine reproductive ultrasonography can, in most operations, be profitable for cattle producers when used by experienced veterinary ultrasonographers.

Once a client has experienced first hand what ultrasound can do, he or she becomes sold on the technology. Much of the guesswork involved with palpation is eliminated, which gives the client complete confidence in a diagnosis, especially when the specific area of interest on the monitor is pointed out to them.

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 thousand 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 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.

Aside from direct income from services rendered, ultrasonography can be a tremendous practice builder. Patients are referred from veterinarians and other clients from surrounding areas for cows to be examined for various problems and fetal sexing. These referrals, almost without exception, come back on a regular basis when ultrasonography is indicated for a diagnosis. Some referred clients have their first exposure with an embryo transfer practitioner and eventually incorporate embryo transfer procedures on their herd. A client's perception of a progressive veterinarian who uses such high-tech diagnostic equipment as an ultrasound unit is greatly enhanced.

The bottom line is that ultrasonography in a bovine practice can be profitable to veterinarians and clients. Veterinarians must understand that the learning curve is time consuming and sometimes frustrating. The initial investment a high-quality ultrasound unit also warrants considerable deliberation. 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 a leader in a relatively new discipline of clinical veterinary medicine, ultrasonography may be the tool to achieve these goals.

Endnotes

[a] *Bovine Reproductive Ultrasonography: A Video Training Tutorial.* Weatherford, TX, 1994. www.biotechproductions.com

[b] *Bovine Fetal Sexing. Unedited tutorial-52 clinical fetal sexing exams on DVD.* www.biotechproductions.com

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