Bovine reproductive ultrasound for practitioners

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Abstract

The use of ultrasound has become common for examination of the female bovine reproductive tract. Early pregnancy and twin diagnosis, confirmation of embryonic and fetal viability, fetal gender determination, identification of fetal anomalies, and accurate assessment of ovarian structures and uterine health are benefits of ultrasound.

Keywords: Ultrasound, pregnancy, embryonic and fetal loss, fetal anomaly, genital tubercle, corpus luteum, follicular cyst, uterine pathology

The embryo and fetus

Under ideal conditions the bovine embryo can be visualized ultrasonographically as early as 21 days after insemination.[1] Under field conditions it is more practical and accurate to wait until at least 27 days in adult cows.[2] Heifers can be scanned for pregnancy with excellent accuracy a day or two earlier.

The embryonic period lasts from one to 42 days of gestation. The risk of pregnancy loss is highest during this period because the placenta has not fully developed. Signs of embryonic death at this point include lack of heartbeat, cloudy amniotic or chorioallantoic fluid, and separation of the chorioallantoic membrane. Twins can be detected by presence of a shared chorioallantoic membrane (twin line), two embryos, and usually two corpora lutea. Over 50% of pregnant cows with two corpora lutea will have twins,[3] unless they have been resynchronized with gonadotropin releasing hormone (GnRH) prior to pregnancy examination. Resynchronized cows will have an even higher percentage of multiple corpora lutea without a concurrent increase in twins.

During the fetal period from 42 days to parturition the differentiation of organs progresses. At approximately 45 days testicular development begins. This is of particular interest in the case of twin pregnancies. If a male fetus dies after this time, but the female twin remains, the female may be a freemartin. By 50 days fetal movement can easily be detected to verify viability.

At 55 days the external genitalia are positioned in their final locations, allowing gender determination until the fetus is beyond reach of the ultrasonographer, sometimes as late as 130 days. The optimal time for many ultrasonographers is 60 to 80 days, when nearly 100% accuracy can be achieved.[4] At this time the genital tubercle is very visible and completely migrated to its final position. Also, the fetus is still within reach and small enough to orient under the transducer.

The genital tubercle will become the penis in the male and the clitoris in the female. Hence, it is located behind the umbilicus and cranial to the thighs in the male and under the tail head and caudal to the thighs in the female. In both genders it usually appears as a hyperechoic bi-lobed structure, although it sometimes appears to have one or three lobes. Teats and scrotum may also be visualized, but it is inadvisable to use these soft tissue structures alone for the diagnosis of fetal gender. Specular reflection artifacts in the umbilicus can look similar to the male genital tubercle.

Embryonic and fetal anomalies

Abembryonic vesicle

An amniotic vesicle is seen either without any visible embryo, or with the embryo outside the vessicles. Such embryos rarely survive even if they have a heartbeat on the day of first examination. Recheck examination usually reveals an empty amniotic sac or complete lack of uterine contents.

Schistosomus reflexus

The fluid-filled fetal stomach can be visualized outside the body cavity. In most cases the spinal column is severely bent, sometimes into a V shape.

Fetal ascites

In a normal fetus the abdominal organs completely fill the cavity with no visible peritoneal fluid. Anechoic areas around the internal organs indicate fetal ascites. In some cases there is also fluid around the heart or lungs. Careful assessment should be made to differentiate fetal ascites from a dead fetus with fluid accumulation.

Fetal anasarca

Fluid is accumulated under the skin rather than in the peritoneal space. Follow-up sometimes reveals that the fluid is no longer visibly present. In other cases there is no change or fetal death occurs.

Umbilical hernia

An abnormally large umbilicus may indicate an umbilical hernia. If the defect is very large the umbilicus may contain tissues of various densities representing abdominal organs.

Amorphous globosus or acardiac twin

These anomlies are differentiated from fetal monsters by lack of a heartbeat and the presence on a normal fetus. These anomalies usually do not grow large so are rarely a problem at parturition. They have no gender so are not of concern for freemartinism.

Conjoined twins

Conjoined twins are very rare and must be differentiated from normal twins lying close together.

Normal vs abnormal ovarian structures

With a good field ultrasound unit the presence of a corpus luteum (CL) can be identified as early as two days after ovulation, and even earlier with a research quality machine. There is a wide variation in the ultrasonographic appearance of normal corpora lutea. Metestrus CLs are more heterogeneous and smaller than diestrus CLs or those of pregnant cows. Up to 80% of CLs have a fluid cavity at some time in their development, usually early. Some cavitary CLs have an echogenic center, probably clotted blood remaining from ovulation. The differentiation of cavitary CLs and cystic CLs is perhaps a matter of academic semantics. This author prefers not to use the term cystic CL unless the structure is proven by repeated examinations to be persistent beyond the normal life of a CL

Follicular cysts have been defined as thin-walled fluid-filled follicular structures greater than 22mm in diameter. A follicular cyst is differentiated from a cavitary CL, luteal cyst, or luteinizing follicular cyst by the absence of a visible luteal rim. Follicular cysts are benign in most cases, with luteal structures and follicular waves developing normally on the same or contralateral ovary. Benign cysts can persist throughout several cycles and into pregnancy. Thorough examination of both ovaries is essential to properly classify follicular cysts. If luteal tissue is detected the cyst is benign. If no luteal tissue is seen a second examination must be performed in about five to fourteen days to determine if a CL has developed. If not, the cyst is likely preventing normal cyclicity.

Ovulation scars are seen as small hyperechoic foci within the ovarian stroma. They tend to be more visible in older cows and embryo donors. These are normal and do not block the entire ultrasound beam as calcifications do.

Granulosa cell tumors are very heterogenic masses usually occupying the entire ovary. They are composed of highly vascularized dense tumor tissue. The acoustic mismatch between the dense neoplastic tissue and the fluid in the blood vessels creates specular reflections throughout the tumor.

Ovarian follicular dysplasia (OFD) has been described.[5] This syndrome begins with bilateral development of clustered solid follicles that eventually progress to a granulosa theca cell tumor. Early cases have the same ultrasonographic appearance as truly anovular or pre-pubertal ovaries. Later in development the ovaries increase in size, showing increased hyperechogenicity, decreased fluid in the follicles, and hyperechogenic shadows due to mineralization of dysplastic follicles.

Uterine and vaginal pathology

In most cases of metritis/endometritis echogenic purulent material can be seen in the uterine lumen. This fluid may be any shade of light gray to very bright white depending on its density. Intraluminal fluid in cases of pyometra is usually voluminous and of medium echogenicity. Cases of chronic subclinical metritis/endometritis usually have little intraluminal fluid of higher echogenicity. Occasionally hyperechoic foci are seen within the endometrium itself, with no intraluminal fluid visible. These foci may be due to bacterial invasion of the tissue from prior pyometra. The ultrasonographer should be careful not to confuse small volumes of echogenic intraluminal fluid with specular reflections due to estrus.

Mucometra is an accumulation of sterile anechoic or slightly echoic fluid in the uterine lumen not associated with pregnancy. Specular reflections may or may not be present. The endometrium is not thickened as it is in estrus. Mucometra does not appear to be related to ovarian structures. It is critical to confirm the absence of an embryo or fetus in cases of mucometra. When in doubt the ultrasonographer should perform a second examination a few days later to confirm the diagnosis. Mucometra in heifers may be related to the presence of a complete hymen or other congenital blockage of the tubal portions of the reproductive tract. The cause in adult cows is unknown.

Uterine lymphosarcoma is identified by very firm thickening of the uterine wall that may be localized or throughout the entire uterus. The affected uterine tissue is homogeneous and dense enough to cause partial shadow artifacts.

Uterine, cervical or vaginal abscesses can be distinguished from metritis, cervicitis or vaginitis by the presence of a capsule around the purulent material. Uterine abscesses have a poor prognosis for future fertility. Cervical and vaginal abscesses may or may not be of concern depending on size and location.

Oviducts are normally difficult to visualize with field ultrasound units. Visible fluid accumulation occurs with salpingitis or blockage.

The normal vagina presents as a thin white line representing the confluence of opposite sides of the vaginal wall. In cases of vaginitis echogenic material is visible in the vaginal vault. Unless echogenic material is also present in the uterus prognosis for fertility is fair to good if a protective sheath is used on the insemination gun at breeding. Vaginitis may be primary or caused by urine pooling. Pneumovagina presents as a reverberation artifact.

Staging the reproductive cycle

In proestrus the uterus becomes more heterogeneous as blood flow increases. Anechoic mucous begins to accumulate in the lumen about three days before ovulation. One, or sometimes more, large (16-20mm) follicles will be present. The CL is usually still visible, but is becoming smaller and more heterogeneous.

During estrus the uterus is most hetergenous. Varying amounts of anechoic mucous are present in the uterine lumen. The large difference in acoustic impedance between the dense endometrium and the fluid mucous often creates specular reflection artifacts on the inner wall of the endometrium. The endometrium thickens to about 2.5 times its normal volume under the influence of estrogen.[6] A mature follicle is present until ovulation at approximately 12 hours after the end of standing heat. Sometimes a portion of the follicular fluid is not expelled during ovulation. In these cases it is difficult to determine if ovulation has occurred. A small, heterogeneous regressed CL may still be seen throughout proestrus and estrus.

After ovulation metestrus begins with the development of a new wave of small follicles (less than 8mm). In the first day or two of the cycle these small follicles are all that is visible on the ovaries. By day two or three a small, heterogenic CL becomes visible, often with an anechoic fluid filled cavity. Occasionally the cavity is filled with very hyperechoic material, probably clotted blood.

During diestrus the uterus is homogeneous and a medium shade of gray. There should be little or no fluid in the lumen. The CL will also be homogeneous and a medium shade of gray. The CL may or may not have a fluid cavity. About 80% of CLs have at a cavity some stage in development, usually early. The cavity may be filled with clear fluid, probably due to incomplete expulsion of follicular fluid

during ovulation. Dense material in the cavity may be clotted blood remaining after ovulation. The fluid cavity tends to fill in over time, but may persist into pregnancy. Progesterone production is dependent on the volume of luteal tissue: the size of a cavity is irrelevant. Follicles will be present in diestrus ranging in size from 2mm to 20mm depending on the stage of follicular wave cycles.

Embryo transfer

Donors should be assessed for uterine health and ovarian function before beginning superovulation protocol. If the cow is examined during follicular recruitment it may be possible to predict the response to future superovulation. Cows that recruit large numbers of follicles at the beginning of each follicular wave can be expected to produce more follicles after follicle stimulating hormone treatment.

It is usually not necessary to examine the donor cow on the day of insemination unless very rare or expensive semen is to be used. In those cases it is helpful to know if enough ovulatory size (8mm or more) follicles are present to warrant insemination. Care must be taken to scan gently over the ovaries without manipulation or pressure to avoid disrupting the bursa.

On collection day ultrasound is much more useful than manual palpation for counting the number of CLs. Small CLs, cavitary CLs and CLs imbedded deep in the ovary can be seen. Some cavitary CLs will have very thin walls and may be difficult to differentiate from unovulated follicles. When these fluid-filled structures are seen the cow should still be flushed. It is possible that more embryos than expected will be retrieved.

Recipients should be evaluated for uterine health and ovarian function prior to beginning estrus synchronization. This is more important for adult cows than for heifers due to increased risk of uterine or ovarian pathology in parous animals. It is critical that ultrasound is used to identify CLs before embryo transfer. Because ultrasound is much more accurate than manual palpation for identifying small or fluid filled CLs more animals will be used. Almost 100% of CLs in recipients are diagnosed with ultrasound, but only 80% with manual palpation.

Synchronization protocols with ultrasound

Earlier pregnancy diagnosis with ultrasound plus higher service rate with synchronization can dramatically increase pregnancy rate. In addition, accurate assessment of ovarian structures improves success of synchronization and reduces unnecessary use of progesterone supplementation. It has been shown that the presence of a CL and the availability of an ovulatory size (8mm or greater) follicle at G1 improve conception rates for Presynch/Ovsynch.[8] Presence of a functional (producing blood progesterone levels of at least 1ng/ml) CL is more important and easier to evaluate than presence of a potentially ovulatory follicle. The ultrasonographer should keep in mind that CLs may be visible in proestrus and metestrus, but are not producing appreciable amounts of progesterone at those times. Careful evaluation of follicular structures and the uterus as described in the section on staging the cycle can assist in determining which animals are at an appropriate stage to begin a protocol.

Ultrasound is also helpful for troubleshooting Presynch failures. When cattle are presynchronized with prostaglandin (Presynch) or Double Ovsynch before beginning Ovsynch all normal, cycling animals should have a CL when the first GnRH injection (G1) is scheduled. An examination of ovaries at this time will reveal if presynchronization was successful. If many cows do not have CLs at this time the protocol has failed. Poor compliance, poor cow health, low body condition scores, nutrition problems, etc., are possible reasons for failure.

If pregnancy examination is done at the appropriate time ovarian structures in open cows can help troubleshoot Ovsynch failures. Normal, cycling open cows bred 28-35 days before examination should have a good CL. If not, there is a problem with management, cow health, compliance, cystic follicles, or embryonic loss. If pregnancy examinations are done prior to 28 days it is more difficult to evaluate the absence of a CL in open cows. They may be cycling normally and are in early metestrus, or they may be anovular.

Resynchronization prior to pregnancy examination has become a popular way to expedite re-insemination of open cows. The most common method is to give GnRH approximately seven days prior to pregnancy examination. Open cows then should be ready for the prostaglandin injection of the protocol, saving seven days. This type of resynchronization at 19 days has been shown to be less effective than at 26 or 32 days,[8] probably because the CL does not have time to mature adequately to become prostaglandin responsive. Adding a second dose of prostaglandin approximately 24 hours after the first may improve regression of the CL in these animals.[9]

Artifacts

Beam width artifact

When one ultrasound beam passes through two tissues of different densities at the same depth a beam width artifact is produced. Two echoes reach the transducer at the same time, but only one is read. These artifacts are most common on the lateral walls of fluid filled structures and cause the sides of the structure to appear more echogenic than the contents.

Crystal defects

Crystal defects are seen as black vertical lines extending vertically from the transducer face. They can be differentiated from shadow artifacts or reflection/refraction artifacts because they always stay in the same location on the monitor and always begin at the face of the transducer. They may be caused by broken crystals in the transducer or by broken wires connecting the crystals to the power source in the unit.

Enhancement artifacts

Enhancement artifacts are a function of machine settings. Because ultrasound beams are attenuated over time and distance it is necessary to increase the gain (volume) at deeper levels so that similar tissues at various depths from the transducer appear the same. However, when less echogenic material such as fluid is near the face of the transducer the beam is not attenuated and structures below the fluid appear brighter than they should.

Mirror image artifacts

Mirror image artifacts are a type of reverberation artifact. They are most common when the pelvic bone lies below the structure being examined. Understanding mirror image artifacts helps avoid mistaking one structure for two.

Reverberation artifacts

Repeated echoing of ultrasound waves between two strong interfaces creates reverberation artifacts. One interface is usually the rectal wall/transducer. The second interface is usually gas or bone. Each time the wave is echoed it is reduced by attenuation. Hence, each reverberated echo is weaker than the previous one. Also, the time delay between echoes results in each later echo being seen farther down on the ultrasound screen. The resulting image on the screen consists of equidistant, increasingly smaller, parallel artifacts.

Shadowing

Material dense enough to block or attenuate the ultrasound beam causes shadowing. Most obvious are shadows caused by bone. Less dramatic shadows can also be caused by manure between the transducer and rectal wall leading to a poor quality dark image.

Reflection and refraction shadows

Fluid reflects and refracts ultrasound waves just as the surface of a lake reflects and refracts light waves. When the ultrasound beam is either refracted at an angle into the fluid or reflected away from the fluid it is not echoed back to the transducer. Hence, no image will be seen below the point of

reflection/refraction. These reflection and refraction shadows are commonly seen at the lateral edges of fluid filled structures.

Specular reflection

Specular reflection artifacts occur when two interfaces of very different densities are aligned horizontally to each other and perpendicular to the ultrasound beam. They are most often seen when a fluid filled structure is adjacent to denser tissue. They appear as thin bright horizontal lines of varying lengths. This artifact is very common in estrus.

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