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# Advances in Bovine Reproductive Ultrasonography

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The advent of diagnostic ultrasonographic technology, particularly the development of real-time imaging and transrectal transducers in the late 1970's, was a hallmark advance for reproductive medicine. With ultrasonography, the veterinarian can visualize organs and structures previously accessible only by rectal palpation. In addition, ultrasonography is quick, easy and noninvasive. As a research tool, ultrasound technology has greatly expanded our understanding of the physiology of ovarian dynamics and uterine events associated with the estrous cycle and pregnancy. As a diagnostic tool, ultrasonography is used extensively for cases of infertility in both the male and female. As a management tool, ultrasound capability is considered to be a necessity or standard for broodmare practice, and the technology is being utilized with ever increasing frequency in cattle breeding management.

## How Ultrasound Works

The principles of ultrasound imaging are relatively simple, being based on the variable ability of different tissues to either reflect or propagate high-frequency sound waves. The relationship between tissue density and image brightness or darkness is similar to that for radiographs. Dense tissue, such as the cervix, reflect more of the sound waves and appear as a brighter image on the screen. Bright images are termed **echogenic**. Conversely, less dense structures such as follicles or intraluminal uterine fluid, absorb sound waves and appear as dark regions on the screen. Dark areas are termed **anechoic**. **Hypoechoic** (darker) and **hyperechoic** (brighter) are relative terms used to describe the echogenicity of tissues in comparison to one another.

## Ultrasound Artifacts

The veterinarian should be aware of a number of common artifacts associated with reproductive ultrasonography which have the potential to confound interpretation of the image. **Distance enhancement** occurs when sound waves strike the far wall of a fluid-filled structure such as a follicle. Echoes from, and tissues behind the far wall appear brighter than echoes from the near wall and adjacent tissues. The image distortion caused by this artifact can be minimized with adjustment of the gain controls. When the sound beam strikes a curved structure at an acute angle, it may bend or refract, causing a lack of echo formation beyond the refraction site. This **refraction artifact** is commonly seen when the beam strikes the sides of fluid-filled follicles. Sound beams that strike the near and far surfaces of a fluid-filled spherical structure at a 90° angle may produce highly echogenic or specular reflection called the **six and twelve o'clock artifact**. Gas-filled bowel loops beneath an area of interest can cause **reverberations** which occur when sound waves are reflected back into the tissue from the transducer. Reverberation causes a second (and sometimes third and fourth) echo to appear below the original image. Reverberation artifact may be

diminished by gain control adjustment, or preferably, by manipulation of the organs so that the gas-filled bowel is no longer below the structure of interest. **Acoustic shadowing** occurs when almost all of the sound beams are reflected at an acoustic interface. Structures beyond the interface cannot be imaged and a shadow is formed. Shadows form when gas-filled bowel loops get between the rectum and the structure of interest, or if the beam strikes very dense tissues such as fetal bones or occasionally, the bovine cervix.

### **Applications in the Bull**

Ultrasonography is noninvasive and can be used both to assess normal function and detect pathologic lesions in the bull. Ultrasonographic examination of the penis, prepuce, testis, epididymis, spermatic cord and accessory sex glands can be used as an adjunct in the routine breeding soundness exam of the bull. Manual palpation, scrotal circumference measurement, and semen evaluation provide important information but may not allow detection of all potentially serious diseases. Ultrasonography is useful in identifying penile lacerations and hematomas, and preputial abscesses. With ultrasound, the veterinarian has the ability to noninvasively differentiate abscesses from hematomas. In contrast to the dynamic nature of the ovary, the male gonad or testis is ultrasonographically static. Therefore, testicular ultrasonography is used primarily to detect abnormality such as a cyst, tumor, abscess, or granuloma. Similar lesions can be detected ultrasonographically in the accessory sex glands, epididymides and spermatic cords. Fluid between the visceral and parietal vaginal tunics of the testis can also be detected ultrasonographically. Due to the testis' homogenous echotexture, the location of the retained testicle in cryptorchid individuals can be determined. In paired structures such as the vesicular glands, an ultrasound exam can accurately measure and compare size and echogenicity between left and right glands.

### **Applications in the Cow**

Using ultrasound, veterinarians can monitor growth and regression of ovarian structures noninvasively and in a sequential manner. The three principle components of ovaries, follicles, corpora lutea (CLs), and stroma, are easy to distinguish on ultrasound. As a research tool, ultrasound has been used to study folliculogenesis. Individual follicles have been mapped and the occurrence of follicular waves has been documented. Ovulation and CL formation can be monitored. From a more practical standpoint, ultrasonography can be used to diagnose cystic ovarian conditions, ovarian abscesses and neoplasms. Follicular cysts have a different ultrasonographic appearance than luteal cysts. Luteal cysts have grey echogenic patches along the inner cyst wall or within the antrum of the cyst. In contrast, follicular cysts have an uninterrupted anechoic antrum with a relatively smooth, thin wall and fewer or no echogenic patches. Ultrasonography can also be used to evaluate ovarian response to treatment for cystic ovaries.

Ultrasound can be used to detect pathologic conditions in the uterus. The uterine wall is thickened with conditions such as endometritis and pyometra. Using ultrasound,

these conditions can be differentiated from mucometra and hydrometra. Mural lesions, including hematomas and abscesses, can be detected and followed with ultrasonography.

Ultrasonography is routinely used in some embryo transfer programs to monitor ovarian status after superovulation treatment. Ultrasound can be used to correlate the number of ovarian CLs with the number of embryos flushed from the donor cow. Ultrasound-guided follicular puncture is being used on an experimental basis to harvest bovine follicular oocytes.

The most common use of ultrasonography in bovine practice is in pregnancy diagnosis and fetal age estimation. One study reports that pregnancy status can be determined with 100% accuracy by day 20. With ultrasonography, normal fetal growth patterns as determined by sonographic fetometry can be established. This information might help veterinarians diagnose irregular fetal growth and choose elective abortion. Imaging the fetal heart allows assessment of fetal viability. Ultrasonography can also be used as an adjunct in the diagnosis of fetal mummification.

A powerful new application of ultrasonography in bovine reproductive management is fetal gender determination. After ultrasonographic diagnosis of fetal sex, cow owners can then make decisions as to management of that particular cow or her pregnancy. Oftentimes for example, breeders desire a specified number of either heifer or bull calves from embryo transfer donors. Fetal sexing can allow the embryo transfer manager to make more timely decisions as to when to change bull selections and when to discontinue collecting embryos from a particular donor. It also allows breeders to market pregnancies of known fetal sex. The remainder of this paper discusses ultrasonographic fetal sex determination in greater detail.

### **Ultrasonographic Fetal Sex Determination in the Bovine and Equine**

**Equipment:** Real-time ultrasonic scanner with a linear-array, transrectal 5 or 7.5 MHz transducer.

**Technique:** Considerable operator experience required.

**Diagnosis of Fetal Sex:** Based upon assessment of the relative location of the **genital tubercle**.

**Anatomy:** The **genital tubercle** is the embryonic structure that differentiates into the penis in males and the clitoris in females. During differentiation the tubercle migrates from its initial position between the hind limbs cranially toward the umbilical cord in males and caudally toward the tailhead in females.

**Ultrasonographic appearance:** The ultrasonographic appearance of the genital tubercle is similar in bovine, equine, male and female fetuses. It is a hyperechoic, bilobed structure; each lobe is elongated dorsoventrally and oval-shaped. This characteristic shape enables it to be recognized despite the presence of other echogenic structures in the vicinity.

The genital tubercle is first identifiable between the hind limbs at ~ 40 days of gestation. Genital tubercle migration occurs between days 40 and 55. By day 55, sex diagnoses can be made in all fetuses.

The **optimal time** for fetal sex determination is **days 59 - 68 in horses**, and **days 55 - 60+ in cattle**. During this time period, the fetus is readily accessible, and the genital tubercle is identifiable and assignable to a "male" or "female" location.

The scrotum is identifiable as an oval-shaped echodense structure located between the hind limbs in male fetuses > 70 days.

In the equine, the genital tubercle appears trilobular in some male and female fetuses by ~ day 80. After day 88, all equine fetuses have trilobular genital tubercles. Curran's study in the bovine ended on day 60 of gestation. In my experience, the genital tubercle in older (> 80 day) bovine fetuses retains its bilobed appearance.

Steps in ultrasonographic imaging:

1. Locate the fetus (not as easy as location by palpation).
2. Determine the orientation of the fetus. Helpful landmarks are:
  - ◆ calvarium with its suture lines
  - ◆ mandibles ("bird beak")
  - ◆ narrowing of rib line at the thoracic inlet
  - ◆ beating heart
  - ◆ umbilicus (uniform diameter and echodensity; seen emanating from membranes and extending to fetus)
  - ◆ limbs (differentiate from umbilicus - heteroechodense, not of uniform diameter; differentiate fore from hind limbs - angulation of joints in longitudinal view, and oval versus a more round trunk shape in fore and hind limbs respectively on X-section)
3. Imaging views:
  - ◆ median plane
  - ◆ dorsal plane
  - ◆ transverse plane

4. Locate the genital tubercle.
  - ◆ male area
  - ◆ female area

**Certainty Scores (Curran):**

99%	both male and female areas examined 3 times, genital tubercle consistently identified in one area
95%	only one area examined 3 times; the other area inadequately inspected, genital tubercle identified
85 - 95%	genital tubercle seen only 2 times; the other area inspected 2 times with no tubercle seen
65 - 80%	one area inspected 1 time, presence or absence of genital tubercle noted
50%	gender not diagnosable

**Accuracy:**

Equine	Diagnosis rate	88% (75/85)
	Accuracy	97% (73/75) overall
		100% (44/44) with 95-99% certainty
Bovine	Diagnosis rate	95% (97/102)
	Accuracy	96% (93/97) overall
		100% (65/65) with 95-99% certainty

**Exam time:**

	<b>Mean</b>
Equine	1'17" (15" - 3'55")
Bovine	1'53" (15" - 8'30")

**So grab a bucket and have at it!**

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