CHAPTER 76

Use of Ultrasonography in Wildlife Species

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When a great new technology is invented, the inventors rarely imagine all the different uses that will be found for their invention in the years to come. The pioneers in the field of ultrasound applications, Sir Francis Galton who developed the ultrasonic whistle at the end of the 19th century or Paul Langevin who used it to detect icebergs in 1917, certainly did not anticipate the many industrial and medical uses of ultrasonography today. By definition, *ultrasound* is any sound with frequencies exceeding the upper limit of the human hearing range (maximum 20 kilohertz [kHz]). In the animal kingdom, the capacity for hearing, or the perception of sound, may be as high as about 200 kHz. Known examples are many nocturnal animals such as bats, moths, and other insects and many marine mammals that use sound to locate their prey.

Starting in the 1930s, the role of ultrasonography as a medical treatment tool was established and, in the 1940s, it was also recognized as a diagnostic tool. Gaining acceptance as a noninvasive imaging technology—and thanks to the many technological improvements that facilitated enhancements in sound waves production, reception, processing, and displaying—ultrasonography has become the second most used (after radiography) imaging technology in medicine today. Whereas radiography is known to have adverse effects resulting from radiation exposure, ultrasonography is deemed free of risks at the energies used for diagnostic purposes.[51] Generally, the use of ultrasound in medical applications is a trade-off between penetration depth and resolution. The higher the frequency of the sound produced, the better is the resolution but the lower is the penetration depth, which makes visualization through thick body layers difficult or impossible. Imaging by ultrasonography is also restricted by its very limited penetration through bone structures or gas and the need for proper coupling of the transducer, as coupling may be limited by external structures such as feathers, scales, or fur.

Ultrasonography gained a foothold in veterinary medicine starting in the late 1950s when it was used to estimate fat and muscle thickness.[71] The first peer reviewed report on such use of the technology appeared in 1961,[70] and the first veterinary report appeared a few years later when the technology was used to evaluate pregnancy in ewes.[41] The technology helps in visualizing shape, structure, and size, and identifies pathologic lesions in many body structures, including skin, muscles, tendons, and internal organs. Ultrasonography has many uses in veterinary medicine, ranging from assessment of body fat[24] to searching for pathologies in soft tissues and tendons, but probably the leading application of this technique today is in reproductive medicine.

Ultrasonography was introduced to the field of reproduction management in humans in the late 1950s, thus opening the way to the exploration and characterization of morphologic, biologic, and pathologic processes and to fertility treatment and intervention under the guidance of ultrasound, thereby revolutionizing the entire field. Despite significant advances in ultrasonographic applications in human and veterinary medicine, adoption of this technology has been slow in zoo and wildlife medicine. Probably the first description of its use as a diagnostic tool in zoo animals was in 1978.[56] For a long time, the use of ultrasonography for reproduction management in nondomestic species was sporadic, but over the years, a growing number of ultrasonographic descriptions of various species have been published. Today, this technique has become an indispensable imaging modality in university veterinary hospitals and private clinics, as well as in the veterinary clinics of many zoos.

Various reasons exist for the slow introduction of ultrasonography in the management of nondomestic animals. By definition, species are different from each other in many aspects, including their morphology and specialized structures. For example, significant differences exist in cardiac anatomy and blood flow among the various taxa. Without detailed knowledge of all species, interpretation of body structures seen in each new species may be challenging. Use of carcasses to conduct comparative studies with ultrasonography and conventional anatomic dissection may benefit all. Although some wild animals (e.g., elephants, rhinoceroses, marine mammals) may be trained to allow examination, in the majority of cases, physical restraint, sedation, or anesthesia are required to facilitate safe examination. Effective penetration of ultrasound is limited to certain depths, which makes it often challenging or practically impossible to visualize various internal structures in large-sized animals (e.g., elephants, whales).

In exotic animals, certain specialized structures or features such as the carapace and plastron in tortoises and turtles, feathers and air sacs in birds, scales on many fish and reptilian species, or exoskeleton in many invertebrates may all interfere with the ultrasonographic examination and may require innovative scanning techniques to overcome at least some of these limiting factors that are usually not encountered in human or mainstream veterinary medicine.[24] The unique features that characterize different taxonomic groups with relation to ultrasonographic examination and the different ways this imaging technique may be used when working with zoo and wild animals belonging to these taxa will be discussed in this chapter. Recent developments in ultrasonography and modifications of commercial system configurations, the decrease in machine size that makes it more portable and affordable, and the new methods of examination will all facilitate increased use of this tool for health and reproduction management in wildlife species. Intraoperative ultrasonographic applications will not be discussed in this chapter, besides their value as a diagnostic tool to improve decision quality during surgery.

FISH

One of the main considerations in ultrasonography is the quality of coupling. When used in human or domestic species, for example, coupling gel is required to exclude air from the interface between the transducer and the body. If the fish remains in water (this is highly recommended to reduce the risk for trauma and stress), the water provides excellent coupling conditions, so coupling gel is not needed. Existence of a large body of water around the fish would also make it possible to scan its body without making actual contact with it, by holding the transducer at a distance of as much as 2 centimeters (cm), depending on the frequency used. Naturally, the transducer must be waterproof or otherwise wrapped in a way that will preclude the risk of exposure to water (e.g., by placing it inside a latex glove). Since electricity is involved, especially in devices that are connected to the electricity grid, every precaution should be taken to ensure safety. The need to keep the fish in water also precludes the use of radiography, which is yet another advantage of ultrasonography.

Small fish are normally sedated for the examination, and large ones may be physically restrained. For large fish, transducers of 2 to 5 megahertz (MHz) are usually chosen, and for small fish, 7.5 to 16 MHz would be more appropriate for better visualization of the small body structures. Some fish and elasmobranchs have scales or calcified integument on their body surface (see Figure 76-1, *D*), and these may interfere with the passage of sound waves, resulting in poor visualization. In many of these species, conducting the examination from the ventral surface may produce better results. When this is not sufficient, and when the animal is large enough, transintestinal ultrasonography may be considered. The tissues of fish (skin, muscle) have higher water content compared with tissues in mammals or birds, and this makes the tissues of fish look somewhat different for practitioners who usually work with mammals. It is also important to remember that the velocity of sound wave transmission through muscle decreases with temperature, whereas that in fat increases, so one may distinguish well between muscle and fat at room temperature, but at about 5° C to 11° C, they may look very similar.[59]

Ultrasonography has been used in the fish industry at least since the early 1980s. The main application of this technology has been for the determination of the sex and maturity status of juvenile and adult fish.[55] This has been done in a wide range of fish species such as Coho salmon (*Oncorhynchus kisutch*), cod (*Gadus morhua*), Atlantic salmon (*Salmo salar*), and Pacific herring (*Clupea harengus pallasi*). Other areas in which ultrasonography has been found to be a useful tool include evaluation of ovarian activity and ovulation,[47] health assessment and diagnosis of pathologies,[65] anatomic studies,[14] and, in aquaculture, muscle development evaluation[4] (see Figure 76-1, *A* through *D*).

Assessment of reproductive potential, sexual maturity, and pregnancy has also been done in elasmobranchs (sharks and rays) such as nurse sharks (*Ginglymostoma cirratum*),[7] thornback ray (*Raja clavata*) and small-spotted cat shark (*Scyliorhinus canicula*),[74] and broadnose sevengill sharks (*Notorynchus cepedianus*).[10] Furthermore, ultrasonography has been used for anatomic description and as a diagnostic tool in various species of sharks. Long-term investigations with ultrasonography may also help elucidate unusual phenomena such as intrauterine cannibalism occurring in lamnid sharks.

AMPHIBIANS

For at least three reasons, amphibians are well suited for ultrasonographic examination: (1) They do not have external structures such as fur, feathers, or scales that may impede imaging; (2) their coelom usually contains some fluid that helps enhance the quality of the images. This fluid may increase in volume in diseased animals (see Figure 76-1, *G*); and (3) to varying degrees, depending on the species, they dwell in water, making it possible to scan them while they are submerged in water, as is done in fish. Because of their body sizes, normally transducers ranging from 5 to 16 MHz are used and are placed directly on the animal's skin, in the water without any direct contact with the animal (Figure 76-2, A), or in contact with the outside of a water-filled plastic container with the animal in it.[68] When the transducer is applied directly to the skin or from outside the plastic container, coupling gel is required to enhance image quality. Restraining the animals may be achieved through sedation, direct manual restraint, or placement of the animal inside a water-filled plastic container that restricts its ability to move. If cold water is used, this will further reduce the mobility of the animal, but caution should be taken not to chill the animal too much.

When studying an amphibian by using ultrasonography, the amphibian heart, which is composed of two atria and a single ventricle, is a good point to start the examination. It is located at the ventral midline between the front limbs and may easily be identified because of its pumping activity. Often, the pericardial space contains a moderate amount of fluid, making visualization better. Amphibians lack a diaphragm, so the liver, which, in healthy animals, would have an appearance similar to that in mammals, could be located next to the heart. The gallbladder, which may be as large as the heart, would appear as an anechoic spherical structure near the heart and the liver. Other abdominal organs such as the stomach, gonads, and urinary bladder may also be visualized[29,68] (see Figure 76-1, *E* and *G*). In both caudates and anurans, especially in those in good body condition, unique fat bodies may be visualized within the coelomic cavity. In anurans, these fat bodies appear as fingerlike projections originating from a common stalk stemming from the base of the gonads. These fat bodies would normally appear to be a bit hyperechoic compared with the liver. In salamanders, these fat bodies have a band shape and may be found between the gonads and the kidneys.

The main applications of ultrasonography in amphibians include assessment of the reproductive status,[38] sex determination,[29] evaluation of cardiac activity and blood flow (including the use of Doppler ultrasonography),[8] and health assessment (e.g., parasitic and neoplastic status) and anatomic studies[29,69] (see Figure 76-1, *E* through *G*).

REPTILES

Reptiles are good candidates for ultrasonography. It may be used either as a stand-alone modality or as a complement to radiography, which is used to clearly image the skeleton and the respiratory system, the two body components that ultrasonography cannot image properly. Most reptile species are relatively docile and may be imaged with minimal physical restraint. Some snakes and lizards, however, are less "cooperative" or are poisonous, and special care should be taken to protect the animal and the handler.

Transcutaneous ultrasonography may be problematic in some reptilian species because of the shell or scales that cover their bodies (e.g., Australian bobtail lizard, *Tiliqua rugosa*; or Komodo dragon, *Varanus komodoensis*). Data are scarce with regard to the normal anatomy and architecture of the coelomic cavity and visceral organs in many reptilian species, as well as the way these structures are viewed in ultrasonographic images, so special care should be taken not to mistake, for example, fat structures for other internal organs. This is especially true because many internal organs in reptiles look quite different from those in mammals. Furthermore, the sizes, shapes, and positions of various internal organs may change, depending on the size of the gastrointestinal and reproductive tracts. When possible, comparative studies between ultrasonography and necropsy are highly recommended. Generally, the lungs in reptiles are positioned dorsal to the other coelomic organs, so ultrasonography is best performed from the lateral or ventral aspect at the region where the lungs are located. In turtles, because of their shell, ultrasonography may be conducted from two restricted windows between the carapace and the plastron. From the front of the animal, between the head, front leg, and thorax, one may visualize the heart, liver, and gallbladder. Preferably a small probe (convex or sector scanner) should be used. From the rear of the animal, next to the pelvic region, the visceral organs, including the kidneys, gonads, urinary bladder, intestines, liver, spleen, and so on, may be viewed. The only exception is the pancake tortoise (*Malacochersus tornieri*), in which direct scanning through the soft plastron is possible. In snakes and lizards, especially those that are covered with thick hard scales, the best ultrasonographic visualization may be achieved from the ventral aspect of the animals. For this purpose, a platform or table with openings in it may be designed so that the animal may be placed over it. This will allow for extended duration of examination with minimal need for restraint of the animal.

Smaller animals are best imaged with 7.5 to 16 MHz transducers, and large ones may be better examined with 2.0 to 5.0 MHz probes. Coupling gel should be applied to achieve proper coupling. In some cases, when the ventral and lateral aspects of the animal are covered with very thick scales or scutes that partially or completely block

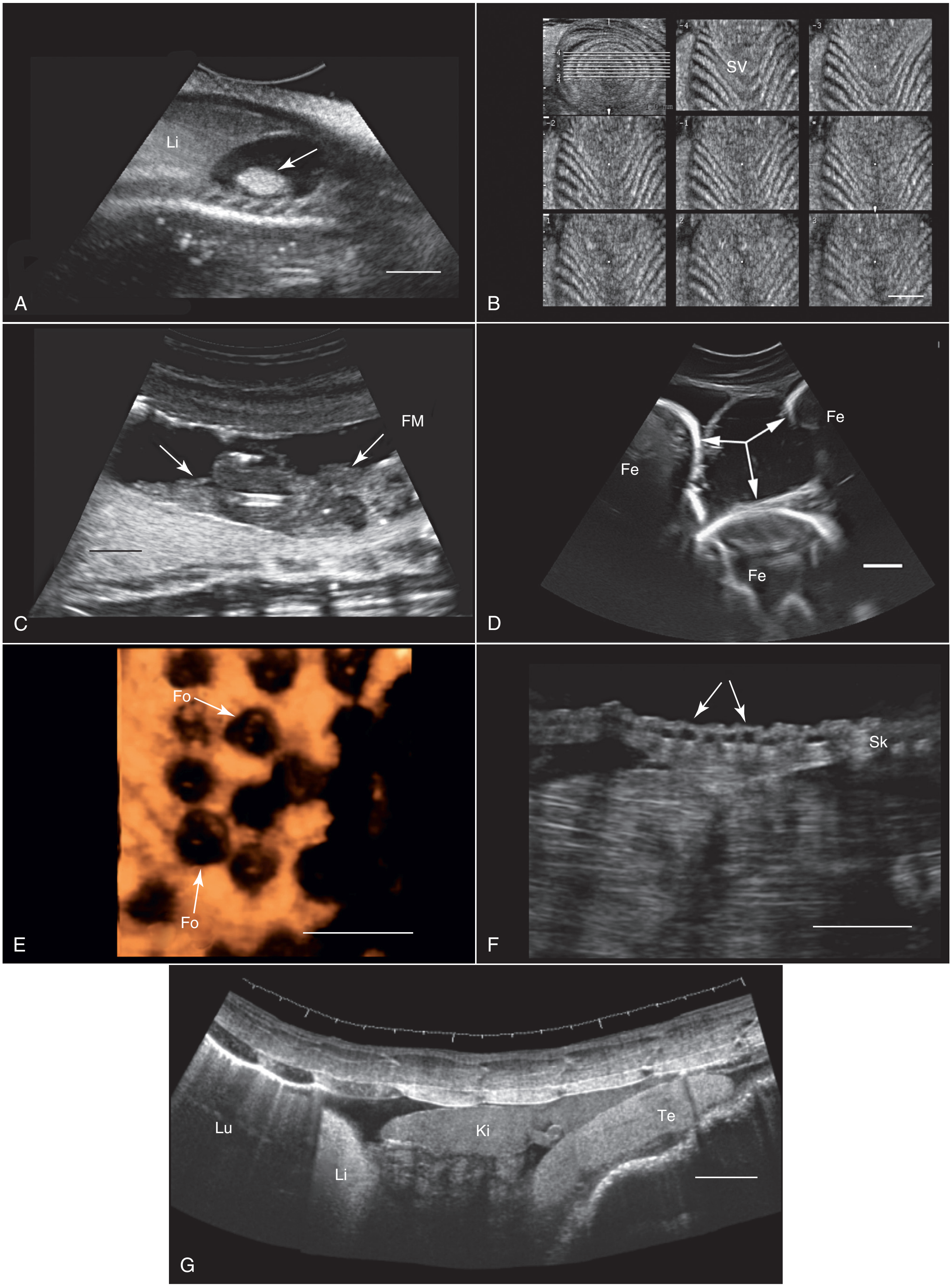
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FIGURE 76-1 **A,** B-Mode sonogram of the liver, and gall bladder that contains a hyperechoic mass (*white arrow*), in a West African lungfish (*Protopterus annectens*). The nature of the lesion (nutritional dysfunction? parasitic product?) has not been diagnosed yet. The wild caught animal was skinnier and smaller than its counterparts. **B,** Three-dimensional ultrasound image of the spiral valve in a black devil stingray (*Potamotrygon leopoldi*) presented in tomographic mode. The unusual sonographic appearance of the modified ileum is normal in rays. Such spiral valves are also found in some shark, skate, and bichir species. As a consequence of the constricting effect the spiral valve has on the lumen of the ileum, rays and sharks cannot pass large hard objects (such as bones) through their lower intestine. Because of its narrow lumen, the spiral valve is often involved in digestive dysfunction in captive elasmobranchs. **C,** B-mode sonogram of the uterine cavity of a black devil stingray (*P. leopoldi*) containing disintegrated fetal material (detritus, *white arrows*) as a result of a failed pregnancy. The caudal part of the liver appears hyperechoic because of the high fat content the liver has in this species. **D,** B-mode sonogram of the uterine cavity of a pregnant lemon shark (*Negaprion brevirostris*) that contained 14 fetuses. Three of them are partially visualized here, along with the fetal membranes (*white arrows*). The surface of the fetal bodies appears surprisingly highly hyperechoic because of the early formation of the typical rough shark skin in these fetuses. The examination was performed directly through the skin on the restraint patient caught in swallow water. **E,** Three-dimensional mode sonogram of an active ovary in an adult female giant salamander (*Andrias davidianus*) containing many large anechoic follicles of approximately 6 millimeters (mm) in diameter with an echogenic center. This reproductive stage is close to egg laying event. **F,** B-mode image of the skin region in an adult male giant salamander (*A. davidianus*). The remarkable fluid-filled skin glands can be easily misinterpreted as follicle formation during sonographic sexing. If a salamander is wrongly handled, it may secrete, within seconds, a sticky, whitish material deriving from these skin glands. **G,** Sonogram of an adult male giant salamander (*A. davidianus*) in panoramic view mode consisting of several single ultrasound images. It allows visualizing a longer region of interest. *From left to right:* The caudal lung field, part of the liver, the elongated kidney, and the cigar-shaped active right testis. A moderate amount of fluid (anechoic region between liver and kidney) is present in the coelomic cavity, which is not unusual in amphibians.
All images were generated using transcutaneous ultrasonography. With the exception of image **C**, all were generated through the water, without direct contact with the animals. Chemical and, in most cases, physical restraint of the patients were not needed. Image **B** was generated using a 6 to 16 megahertz (MHz) transducer, and for all other images, a 2 to 5 MHz transducer was used. White or black bar represents 20 mm.*Fe*, fetus; *FM*, fetal mass; *Fo*, follicle; *Ki*, kidney; *Li*, liver; *Lu*, lung; *Sk*, skin; *SV*, spiral valve; *Te*, testis.

ultrasound waves (e.g., *Tiliqua* spp., *Corucia zebrata*), coupling gel may be applied and left for 15 to 30 minutes so that the scales absorb some of the gel for better imaging. The alternative is to submerge the region of interest (excluding the head) in water to improve coupling. Placing the probe between the scales or from a more lateral position may also help. In larger reptiles, a good alternative would be to use specialized transducers that allow for transintestinal endosonography (see Figure 76-2, *C*). By inserting the transducer through the cloaca into the intestine, the entire urogenital tract, intestines, adrenal, and fat bodies may be visualized.[27]

The primary use of ultrasonography in reptiles is probably for the assessment of the reproductive tract and reproductive status (see Figure 76-2, *B*, *C*, and *E*). This was done in a wide variety of terrestrial, marine, and fresh-water reptilian species.[39,60,62] Sex determination in sub-adult or monomorphic reptiles has also been done with the use of ultrasonography, for example, in komodo dragons (*Varanus komodoensis*), white-throated monitors (*Varanus albigularis*), Gila monsters (*Heloderma suspectum*), and beaded lizards (*H. horridum*).[27,52] Ultrasonography has also proven useful for medical diagnosis [45] (see Figure 76-2, *D*), as well as for ultrasound-guided transcutaneous biopsy.[34] Ultrasonography is very useful for anatomic, morphologic, and nutritional studies in reptiles.[27,64]

BIRDS

Birds are normally covered with feathers that have a large volume of air within them. Even with the use of coupling gel, this may hinder clear depiction of the different internal structures. The existence of large air sacs, the very compacted intestines, and the subcutaneous accumulation of fat, as well as the follicles of the feathers may all further impede transmission of the sound waves. For these reasons, transcutaneous application of ultrasonography is of limited value in birds compared with other vertebrates. When transcutaneous ultrasonography does not provide clear images, transcloacal[23] or transintestinal[15] ultrasonography, using high-resolution miniaturized probes, may be considered. The entire urogenital system, the gonads, and the adrenals may be viewed by the transintestinal technique, and only the caudal part of the genital tract may be viewed by the transcloacal technique (Figure 76-3, A). Identifying the inactive ovaries and visualizing the kidneys, which are located next to the vertebral column and hidden by the compacted intestines and the air sacs, may be difficult at times.

In larger species of birds such as penguins, it is also possible to view much of the abdominal cavity by inserting a small ultrasound probe through the esophagus and conducting nonsurgical transgastric ultrasonography (see Figure 76-2, *G* and *H*). This new transgastric examination technique allows fast and accurate evaluation of large parenchymatous organs such as the lungs, liver, spleen, and kidneys with ultrasonography. Such examination is relevant, for example, when aspergillosis is suspected in penguins. In the very large birds such as the ostrich (*Struthio camelus*) or brown kiwi (*Apteryx mantelli*), ultrasonography may easily be used transcutaneously to monitor ovarian activity and development of ova and to view internal organs such as the heart, intestines, liver, and kidneys.[35,72] The gallbladder is available as an anechoic round or oval-shaped landmark dorsal to the right liver lobe in most birds but not in Psittacines and Columbiformes.

To view internal organs by using transcutaneous ultrasonography, the feathers at the ventral midline, just caudal to the sternal keel and cranial to the pubic bones, are parted, and coupling gel is applied.

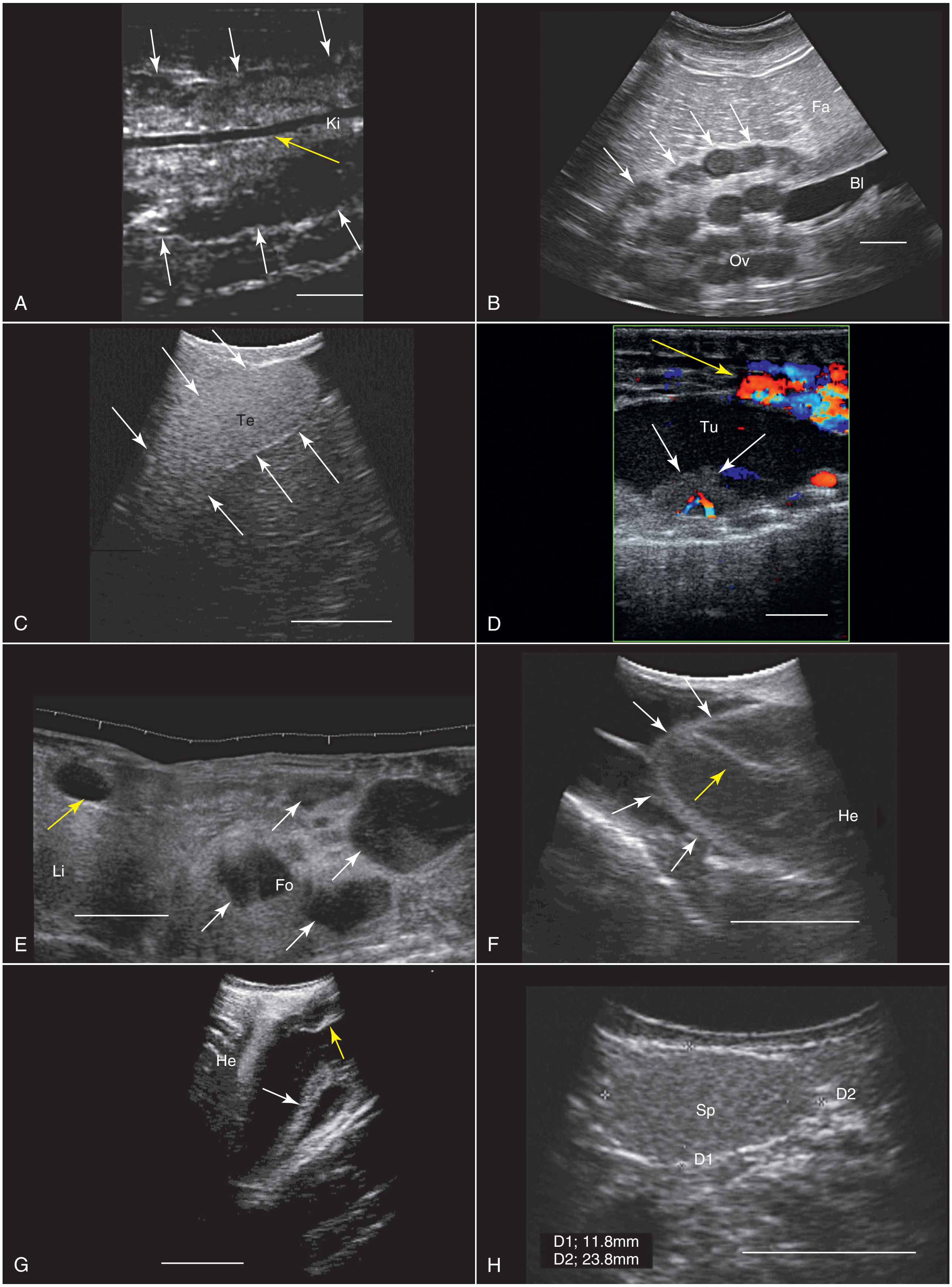

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FIGURE 76-2 **A,** Transcutaneous B-mode image (2 to 5 megahertz [MHz]) of the elongated kidneys (*border marked by white arrows*) separated by a central blood vessel (*yellow arrow*) in an adult hellbender (*Cryptobranchus alleganiensis*). Both kidneys show clear signs of gout (*irregular white dots*) in their parenchyma. The sonogram was generated through the water, without direct contact with the animals. Physical or chemical restraints of the patient were not needed. **B,** Transcutaneous ultrasound image (2 to 5 MHz) showing early development of ovarian follicles in a female Komodo dragon (*Varanus komodoensis*). The partially filled urinary bladder borders the ovary from the caudal aspect. **C,** Because of the ossified scales in the Komodo dragon (*V. komodoensis*), it is not possible to visualize the testes by transcutaneous ultrasonography in this species. The only option for imaging the cigar-shaped testes, located cranioventral to the kidneys, is by transintestinal ultrasonography using a miniaturized transducer (fingertip probe, 7.5 MHz). **D,** Sonogram in color-flow-Doppler mode (6 to 16 MHz, transcutaneous) showing a productive tumor (*white arrows*) with own blood supply, diagnosed in an inland taipan (*Oxyuranus microlepidotus*). The fluid-producing lesion (hypoechoic area above solid tumor tissue) is located near the heart base (*yellow arrow*). **E,** Panoramic view mode sonogram (6 to16 MHz, transcutaneous) showing the coelomic region in a female tuatara (*Sphenodon punctatus*) with an active ovary (*white arrows*). Yellow arrow marks the fluid-filled gall bladder. The patient was physically restrained for the ultrasound assessment. **F,** Transcutaneous B-mode sonogram (7.5 MHz) of a diseased heart in a tauraco (*Tauraco leucolophus*). The coelomic cavity is filled with anechoic transudate framing the apex of the heart (*white arrows*). The left and right ventricles are dilated. The yellow arrow points toward the intraventricular septum. Coelomic cavity aspiration was performed under ultrasound guidance to diagnose the origin of the coelomic effusion. **G,** B-mode sonogram (7.5 MHz) of the four-chamber heart view in an anesthetized rockhopper penguin (*Eudyptes chrysocome*) generated by transgastric technique, in which a miniaturized transducer (fingertip probe) is inserted through the open beak into the empty stomach. The white arrow points to the interventricular septum and the right ventricle. The yellow arrow indicates the aortic valve at the heart base. **H,** B-mode sonogram (7.5 MHz) of the egg-shaped spleen in an anesthetized rockhopper penguin (*E. chrysocome*), generated by transgastric ultrasonography. The organ capsule appears hyperechoic, and the parenchyma appears homogeneous and moderately echogenic. The dimension and echographic appearance indicate a healthy organ.

In all images, the white bar represents 20 millimeters (mm).

Bl, bladder; *Fa*, fat; *Fo*, follicle; *He*, heart; *Ki*, kidney; *Li*, liver; *Ov*, ovary; *Sp*, spleen; *Te*, testis; *Tu*, tumor.

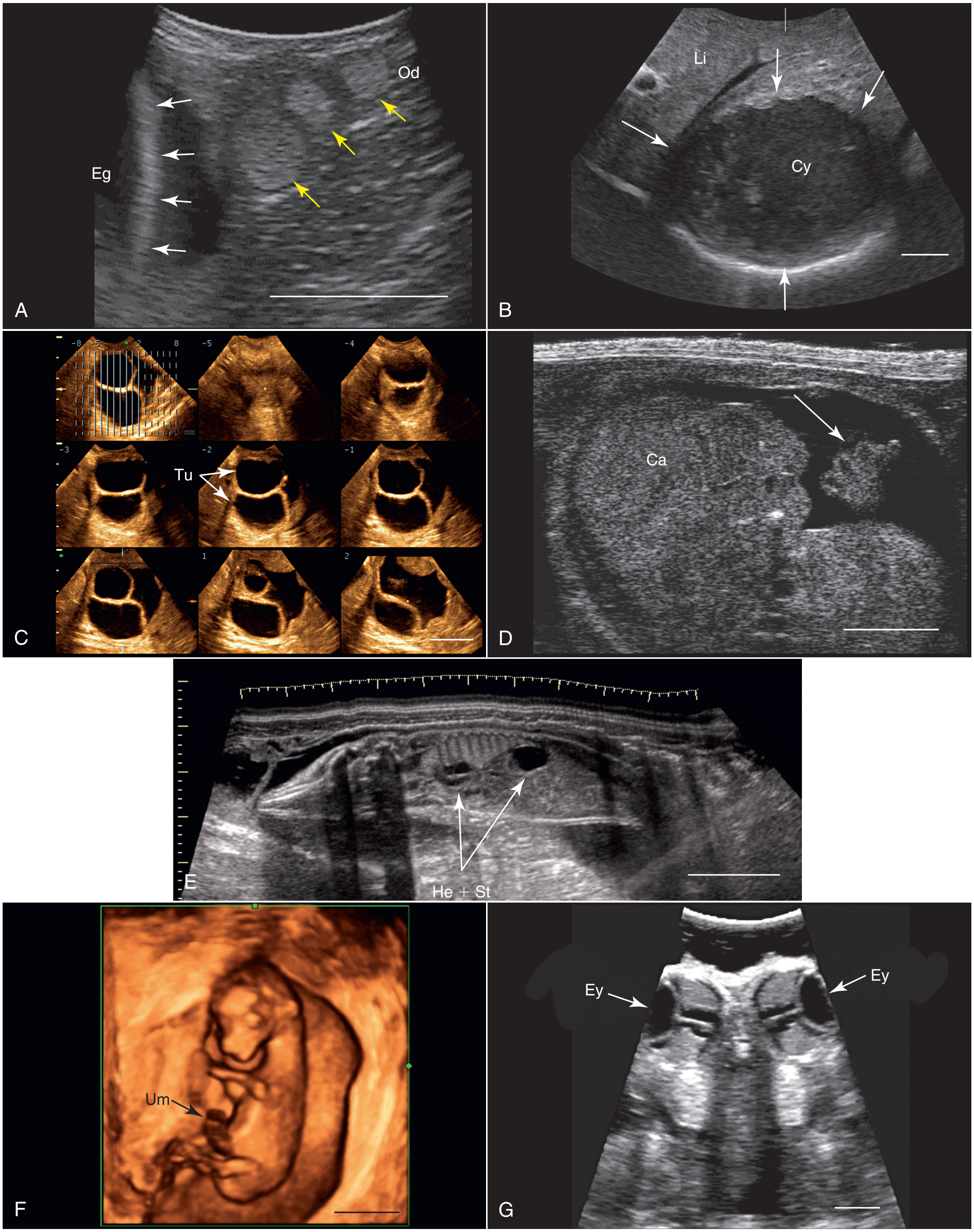
The ventral plumage may also be plucked, when needed, to improve coupling. In small bird species, transducers of 7.5 to 16 MHz are used, and 2 to 5 MHz transducers are useful in the large birds. In some species, the available window may be very limited because of caudally extended keel (e.g., Galliformes and Anseriformes). In such cases, a more lateral approach, placing the transducer just caudal to the last rib on the right side, may provide some view of the internal organs. Fasting the birds for several hours (birds of prey even for a day or two) may enhance clarity of the images. Especially in small and unhealthy birds, the cooling effect the coupling gel may have on the bird as well as the stress and respiratory distress that restraint, positioning, and handling may have on the animal are important considerations.

Some of the uses of ultrasonography in birds include monitoring of muscle development and changes with activity and season,[42] ultrasound-guided fine-needle aspiration[54] (see Figure 76-2, *F*), medical evaluation of internal organs,[58] estimating bone mineral density using amplitude-dependent speed-of-sound quantitative ultrasonography and detection of bone pathologies,[13,53] determination of sex in monomorphic species,[23] evaluation of ovarian status,[50] study of the anatomy and morphology of organs,[19] monitoring the development of the cardiovascular system,[49] and endocardiography.[57] In addition, use of ultrasonography for high-resolution eye examination (20 MHz) in raptors and large birds may be helpful for verification of the impact of trauma and identification of neurologic symptoms.

MAMMALS

Almost two decades ago, the vast majority of publications on ultrasonography use in vertebrates were focused on mammals.[17] This is most probably still the case today. Mammals vary significantly in size, ranging from the 1.8-gram (g) Etruscan shrew (*Suncus etruscus*) to the 170-ton blue whale (*Balaenoptera musculus*), and live in a wide variety of environments, that is, air (e.g., bats), water (e.g., marine mammals), and land. In the over 5400 extant mammalian species, a wide variety of anatomic features have evolved, making knowledge gained on one species not always applicable to another in a straightforward manner. The ideal, which is still lacking for many species, is to have a clear anatomic picture of the species involved before attempting to apply ultrasonography. In the absence of such anatomic knowledge or past experience, identification of pathologic changes may not always be possible.

The vast differences among species dictate the selection of ultrasonography technique to be applied and the quality of images obtained. Some species or individuals may be trained to allow ultrasonographic examination without the need for sedation or anesthesia. These include many marine mammals, primates, elephants, and rhinoceroses, among others. Many mammals have fur cover over their bodies. To achieve good coupling during transcutaneous examination, the fur is ideally clipped over the area of interest. If clipping the fur is not an option, soaking it with alcohol and then applying coupling gel may also help obtain acceptable results. The fur in marine mammals is a very important part of their insulation layer, so it is best not to clip it unless absolutely necessary. In such cases, when high-quality transcutaneous imaging cannot be achieved, transrectal examination may provide excellent results for much of the visceral region. Many other marine mammals and a few other mammals such as the naked mole rat (*Heterocephalus glaber*), the naked bat (*Cheiromeles torquatus*), and the common (*Hippopotamus amphibious*) and pygmy (*Choeropsis liberiensis*) hippopotamuses have skin almost devoid of hair, which makes scanning easy. Marine mammals are sensitive to ultrasonic waves and may be trained to accept ultrasonographic examination in water. Their examination



may thus be conducted in a manner similar to that in fish, keeping in mind the size and anatomic differences.

Skin thickness in animals such as rhinoceroses, hippopotamuses, or elephants and fat pads in many marine mammals limit the use of transcutaneous ultrasonography in these animals. The very large body sizes of some mammals also limit the ability to visualize some internal structures, even when using the transrectal ultrasonography approach. Development of special tools and extensions may help,[30] but even these have their limitations with regard to imaging the deep structures in the body. At the other end, the very small mammals

FIGURE 76-3 **A,** B-mode sonogram (7.5 megahertz [MHz]) of the oviduct in a Humboldt penguin (*Spheniscus humboldti*) visualized by transintestinal ultrasonography via the cloaca. White arrows point at the caudal part of a forming egg surrounded by anechoic oviductal fluid. Yellow arrows indicate three cross-sections of the activated and folded oviduct. **B,** Transcutaneous ultrasound image (B-mode, 2 to 5 MHz) of the central liver lobe of a chimpanzee (*Pan troglodytes*). The lobe is severely altered by an echinococcosis. White arrows mark the border of the parasitic cyst formation; the content in the hydatid cyst has an irregular appearance because of the large number of protoscoleces in it. **C,** Transvaginal three-dimensional ultrasound image presented in tomographic mode (5 to 9 MHz). The sonogram shows an aggressive ovarian tumor in a female Western lowland gorilla (*Gorilla gorilla gorilla*) with several fluid-filled chambers (*white arrows*). **D,** High-frequency ultrasound image (B-mode, 55 MHz) generated with an "ultrasound biomicroscope," which operates with transducer frequencies of up to 70 MHz. The resolution allows counting the fingers (*white arrow*) of a 4-week-old naked mole rat fetus (*Heterocephalus glaber*) in this transcutaneous sonogram. **E,** Panoramic-view-mode sonogram consisting of several single ultrasound images, allowing visualization of a longer region of interest. The image shows a 2-month-old bottlenose dolphin (*Tursiops aduncus*) fetus (2 to 5 MHz, transcutaneous). The calcified fetal skull is causing shadow artifacts. White arrows mark heart and fluid-filled stomach. **F,** Three-dimensional mode sonogram (2 to 5 MHz, transcutaneous) of a 7-week-old Beira antelope (*Dorcatragus megalotis*) fetus. **G,** B-mode sonogram (3.5 MHz) of the eyes region (*white arrows*) in a North Pacific giant octopus (*Enteroctopus dofleini*). Note the clearly visible nervus opticus running from the eyes into the central brain. The image was generated through the water without direct contact with the animal. Physical or chemical restraints of the patient were not needed. In **D,** the white bar represents 2 millimeters (mm), and in all other images, the white or black bar represents 20 mm.

Ca, cranium; *Cy*, cyst; *Eg*, egg; *Ey*, eye; *He*, heart; *Li*, liver; *Od*, oviduct; *St*, stomach; *Tu*, tumor; *Um*, umbilicus.

may be easily studied with normal transducers of 7.5 to 16 MHz or with ultrasound biomicroscopes of 50 to 70 MHz, which may provide excellent images at a resolution of 30 micrometers (μm) or less[61] (see Figure 76-3, *D*). In elephants, rhinoceroses, giraffes, and some other large terrestrial mammals, ultrasonography is usually performed with the animal in the standing position with the use of training of the animal to accept the examination or with sedation. In smaller species, examination would be ideally performed with the animal in dorsal recumbency, which allows easy access to the abdominal area. For cardiac imaging, it is best to turn the animal to left recumbency, with the probe being moved from under the animal, facing up.

The early application of medical ultrasonography in animals was for pregnancy diagnosis in mammals.[41] Today, the leading application for ultrasonography is still in the area of reproduction in wildlife mammalian (and other) species (see Figure 76-3, *C* through *F*). It is used to monitor the urogenital tract in both males[31] and females;[28] identify reproductive tract pathologies;[22] characterize the female reproductive status and elucidate the reproductive cycle pattern through longitudinal studies;[43] assess male reproductive status and collect semen;[26,30] help determine the sex of the fetus[9] as well as that of adult animals in the few monomorphic mammalian species such as beavers, sloths, and the spotted hyena;[33] and determine and monitor pregnancy and fetal development and diagnose embryonic resorption in various species, including the giant panda (*Ailuropoda melanoleuca*), Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants, snow leopard (*Uncia uncia*), bonobos (*Pan paniscus*), European brown hare (*Lepus europaeus*), and tammar wallaby (*Macropus eugenii*).[11,12,16,25,44,66] Beyond reproductive assessment and monitoring, ultrasonography is highly useful when it comes to the application of assisted reproductive technologies, many of which are performed under the guidance of ultrasound. Ultrasonography plays a paramount role in procedures such as ovum pickup, artificial insemination, and embryo transfer in various species.[21,32]

Ultrasonography is also used in nondomestic mammals for diseases diagnosis and identification of pathologic processes in species such as the cheetah (*Acinonyx jubatus jubatus*),[73] koala (*Phascolarctos cinereus*),[48] and oncilla (*Leopardus tigrinus*).[3] As a specialization within ultrasonography, echocardiography is performed in both adults[63] and fetuses[67] to evaluate the health of the cardiovascular system. Ultrasonography has been used in anatomic studies[2] and for assessing body fat (and, thus, nutritional status) since the 1950s in the cattle and swine industries.

Marine mammals may be considered a specialized group within the mammalian class. The group includes the cetaceans, sirenians, pinnipeds, and fissipeds, the first two being fully aquatic and the last two being semi-aquatic in nature. Handling of the semi-aquatic species for ultrasonographic examination is similar to that of other terrestrial mammals. Many of the seals may be trained to lie on land for the examination, or they may be physically restrained relatively easily for the duration of the examination. Some of the aquatic species may also be trained to present themselves for examination and, at times, also to come on land. Because of the sensitivity of many of these species to ultrasound waves, care should be taken to avoid contact between the transducer and the water, unless the animal has been specifically trained to accept this. As in their terrestrial counterparts, ultrasonography has been used in marine mammals to assess reproductive status in both males [6] and females,[1] study early embryonic development[1] (see Figure 76-3, *E*), identify fetal malformations,[5] and for medical diagnosis.[36] Ultrasonographic evaluation of body condition and nutritional status is performed through measurements of the dermis, epidermis, and blubber. This has been performed in a wide variety of species, including Steller sea lions (*Eumetopias jubatus*), harbor seals (*Phoca vitulina*), and Southern elephant seals (*Mirounga leonine*).

INVERTEBRATES

By definition, *invertebrates* are animals that are not included in the vertebrata subphylum. Generally, very little work has been done and published on the use of ultrasonography in invertebrates. When imaging water-dwelling invertebrates, ultrasonography may be used with the animal submerged in the water, as in the case of fish. Here, too, direct contact of the transducer with the animal's body is not an absolute must (see Figure 76-3, *G*). Because of their relatively small body size, transducers of 5 MHz and above, up to 20 MHz, for normal ultrasonography and further to the ultrasound biomicroscope range of 50 to 70 MHz, are normally used for most species.

As invertebrates lack bones, no interference with ultrasound transmission occurs. However, many invertebrate species (e.g., insects, crustaceans) have an exoskeleton, which significantly limits the available windows for viewing visceral organs. In one study in crustaceans (iridescent swimming crab, *Portunus gibbesii*), the heart and scaphognathites could be easily detected, but other internal organs could not be properly visualized.[20] If exoskeleton is absent, anatomic studies of various body parts may be conducted. Ultrasonography has been used, for example, to study the morphology of the arms,[46] or the brain,[18] in the common octopus (*Octopus vulgaris*) or blood circulation in cuttlefish (*Sepia officinalis*).[37] The heart in invertebrates may also be studied by using Doppler ultrasonography.[40]

CONCLUSION

Advances in ultrasound technology have turned it into a highly accessible, relatively inexpensive, and, in many models, portable imaging tool. Keeping in mind the vast anatomic differences among and within the various taxonomic groups, the technology may be used in the study and management of zoo and wildlife animals in all vertebrates and, to a lesser extent, in invertebrates. Ultrasonography has a wide range of applications related to reproduction assessment and assisted reproduction technologies, disease diagnosis, and physiologic, anatomic, and morphologic studies. Being a real-time, noninvasive and practically risk-free imaging technique, ultrasonography has many advantages over radiography, particularly when soft tissue is the target. The veterinary literature, however, is still lacking in detailed reports on the use of ultrasonography in different taxa. This will certainly change in time as the use of ultrasonography in nondomestic species expands rapidly.

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REFERENCES

1. Adams GP, Ward Testa J, Goertz CEC, et al: Ultrasonographic characterization of reproductive anatomy and early embryonic detection in the northern fur seal (*Callorhinus ursinus*) in the field. *Marine Mammal Sci* 23(2):445–452, 2007.
2. Bapodra P, Bouts T, Mahoney P, et al: Ultrasonographic anatomy of the Asian elephant (*Elephas maximus*) eye. *J Zoo Wildl Med* 41(3):409–417, 2010.
3. Bignardi Jarretta G, Bombonato PP, de Barros Vaz Guimarães MA: Renal ultrasonographic evaluation in the oncilla (*Leopardus tigrinus*). *J Zoo Wildl Med* 35(3):356–360, 2004.
4. Bosworth BG, Holland M, Brazil BL: Evaluation of ultrasound imagery and body shape to predict carcass and fillet yield in farm-raised catfish. *J Anim Sci* 79(6):1483–1490, 2001.
5. Brook F: Ultrasound diagnosis of anencephaly in the fetus of a bottlenose dolphin (*Tursiops aduncas*). *J Zoo Wildl Med* 25(4):569–574, 1994.
6. Brook FM, Kinoshita R, Brown B, et al: Ultrasonographic imaging of the testis and epididymis of the bottlenose dolphin, *Tursiops truncatus aduncas*. *J Reprod Fertil* 119(2):233–240, 2000.
7. Carrier JC, Murru FL, Walsh MT, et al: Assessing reproductive potential and gestation in nurse sharks (*Ginglymostoma cirratum*) using ultrasonography and endoscopy: An example of bridging the gap between field research and captive studies. *Zoo Biol* 22(2):179–187, 2003.
8. Coucelo J, Coucelo J, Azevedo J: Ultrasonography characterization of heart morphology and blood flow of lower vertebrates. *J Exp Zool* 275(2–3):73–82, 1996.
9. Curran S, Ginther OJ: Ultrasonic diagnosis of equine fetal sex by location of the genital tubercle. *J Equine Vet Res* 9:77–83, 1989.
10. Daly J, Gunn I, Kirby N, et al: Ultrasound examination and behavior scoring of captive broadnose sevengill sharks, *Notorynchus cepedianus* (Peron, 1807). *Zoo Biol* 26(5):383–395, 2007.
11. Drews B, Hermes R, Göritz F, et al: Early embryo development in the elephant assessed by serial ultrasound examinations. *Theriogenology* 69(9):1120–1128, 2008.
12. Drews B, Roellig K, Menzies BR, et al: Ultrasonography of wallaby prenatal development shows that the climb to the pouch begins in utero. *Sci Rep* 3:1458, 2013.
13. Fleming RH, Korver D, McCormack HA, et al: Assessing bone mineral density in vivo: Digitized fluoroscopy and ultrasound. *Poult Sci* 83(2):207–214, 2004.
14. Goddard PJ: Ultrasonic examination of fish. In Goddard PJ, editor: *Veterinary ultrasonography*, Wallingford, U.K., 1995, Cab International, pp 289–302.
15. Göritz F, Hildebrandt TB, Hermes R, et al: Immobilisation and transintestinal sonography in cassowary. In *Verhandlungsbericht des 38 Internationalen Symposiums über die Erkrankungen der Zoo- und Wildtiere*, Zurich, Switzerland, 1997, pp 181–186.
16. Göritz F, Hildebrandt TB, Jewgenow K, et al: Transrectal ultrasonographic examination of the female urogenital tract in nonpregnant and pregnant captive bears (Ursidae). *J Reprod Fertil Suppl* 51:303–312, 1997.
17. Göritz F: *Sonographie bei Zoo- und Wildtieren. D.V.M. Dissertation Thesis*, Berlin, Germany, 1996, Free University.
18. Grimaldi AM, Agnisola C, Fiorito G: Using ultrasound to estimate brain size in the cephalopod *Octopus vulgaris Cuvier* in vivo. *Brain Res* 1183:66–73, 2007.
19. Gumpenberger M, Kolm G: Ultrasonographic and computed tomographic examinations of the avian eye: Physiologic appearance, pathologic findings, and comparative biometric measurement. *Vet Radiol Ultrasound* 47(5):492–502, 2006.
20. Haefner PA, Jr: Application of ultrasound technology to crustacean physiology: Monitoring cardiac and scaphognathite rates in Brachyura. *Crustaceana* 69(6):788–794, 1996.
21. Hermes R, Göritz F, Portas TJ, et al: Ovarian superstimulation, transrectal ultrasound-guided oocyte recovery, and IVF in rhinoceros. *Theriogenology* 72(7):959–968, 2009.
22. Hermes R, Hildebrandt TB, Walzer C, et al: The effect of long non-reproductive periods on the genital health in captive female white rhinoceroses (*Ceratotherium simum simum*, *C.s. cottoni*). *Theriogenology* 65(8):1492–1515, 2006.
23. Hildebrandt T, Pitra C, Sömmer P, et al: Sex identification in birds of prey by ultrasonography. *J Zoo Wildl Med* 26(3):367–376, 1995.
24. Hildebrandt TB, Göritz F: Use of ultrasonography in zoo animals. In Fowler ME, editor: *Zoo and wild animal medicine*, Philadelphia, PA, 1998, Saunders, pp 41–54.
25. Deleted in proof.
26. Hildebrandt TB, Brown JL, Göritz F, et al: Ultrasonography to assess and enhance health and reproduction in giant panda. In Wildt DE, Zhang A, Zhang H, et al, editors: *Giant pandas: Biology, veterinary medicine and management*, Cambridge, U.K., 2006, Cambridge University Press, pp 410–439.
27. Hildebrandt TB, Göritz F, Pitra C, et al: Sonomorphological sex determination in subadult Komodo dragon. In *Proceedings of the annual conference of the American Association of Zoo Veterinarians*, Puerto Vallarta, Mexico, 1996, pp 251–254. (abstract).
28. Hildebrandt TB, Göritz F, Pratt NC, et al: Ultrasonography of the urogenital tract in elephants (*Loxodonta africana* and *Elephas maximus*): An important tool for assessing female reproductive function. *Zoo Biol* 19(5):321–332, 2000.
29. Hildebrandt TB, Göritz F, Schaftenaar W, et al: Sonomorphologische Geschlechtsbestimmung und Einschätzung der reproduktiven Kapazität bei Riesensalamandern (Cryptobranchidae). In *Verhandlungsbericht des 38 Internationalen Symposiums über die Erkrankungen der Zoo- und Wildtiere*, Zurich, Switzerland, 1997, pp 175–180.
30. Hildebrandt TB, Hermes R, Jewgenow K, et al: Ultrasonography as an important tool for the development and application of reproductive

technologies in non-domestic species. *Theriogenology* 53(1):73–84, 2000.

31. Hildebrandt TB, Hermes R, Pratt NC, et al: Ultrasonography of the urogenital tract in elephants (*Loxodonta africana* and *Elephas maximus*): An important tool for assessing male reproductive function. *Zoo Biol* 19(5): 333–345, 2000.
32. Hildebrandt TB, Hermes R, Saragusty J, et al: Enriching the captive elephant population genetic pool through artificial insemination with frozen-thawed semen collected in the wild. *Theriogenology* 78(6):1398–1404, 2012.
33. Hildebrandt TB, Pitra C, Göritz F, et al: Sonomorphologische Geschlechtsdiagnose bei der Tüpfelhyäne (*Crocuta crocuta* Erxleben). *Fertilität* 12:46–50, 1996.
34. Isaza R, Ackerman N, Schumacher J: Ultrasound-guided percutaneous liver biopsy in snakes. *Vet Radiol Ultrasound* 34(6):452–454, 1993.
35. Jensen T, Durrant B: Assessment of reproductive status and ovulation in female brown kiwi (*Apteryx mantelli*) using fecal steroids and ovarian follicle size. *Zoo Biol* 25(1):25–34, 2006.
36. Kenny DE, Irlbeck NA, Eller JL: Rickets in two hand-reared polar bear (*Ursus maritimus*) cubs. *J Zoo Wildl Med* 30(1):132–140, 1999.
37. King AJ, Henderson SM, Schmidt MH, et al: Using ultrasound to understand vascular and mantle contributions to venous return in the cephalopod *Sepia officinalis* L. *J Exp Biol* 208(11):2071–2082, 2005.
38. Kouba AJ, Vance CK: Applied reproductive technologies and genetic resource banking for amphibian conservation. *Reprod Fertil Dev* 21(6):719–737, 2009.
39. Lance VA, Rostal DC, Elsey RM, et al: Ultrasonography of reproductive structures and hormonal correlates of follicular development in female American alligators, *Alligator mississippiensis*, in southwest Louisiana. *Gen Comp Endocrinol* 162(3):251–256, 2009.
40. Lewbart GA, Mosley C: Clinical anesthesia and analgesia in invertebrates. *J Exotic Pet Med* 21(1):59–70, 2012.
41. Lindahl IL: Detection of pregnancy in sheep by means of ultrasound. *Nature* 212(5062):642–643, 1966.
42. Lindström A, Kvist A, Piersma T, et al: Avian pectoral muscle size rapidly tracks body mass changes during flight, fasting and fuelling. *J Exp Biol* 203(5):913–919, 2000.
43. Lueders I, Niemuller C, Gray CS, et al: Luteogenesis during the estrous cycle in Asian elephants (*Elephas maximus*). *Reproduction* 140(5):777–786, 2010.
44. Lueders I, Niemuller C, Rich P, et al: Gestating for 22 months: luteal development and pregnancy maintenance in elephants. *Proc R Soc B Biol Sci* 279(1743):3687–3696, 2012.
45. Luz S, Dorrestein GM, Zwart P, et al: Management, diagnosis and treatment of an inland taipan (*Oxyuranus microlepidotus*) with a tumor-like swelling in the heart region at Singapore Zoological Garden. In *The International Conference on Diseases of Zoo and Wild Animals*, Beekse Bergen, The Netherlands, 2009, pp 33–42.
46. Margheri L, Ponte G, Mazzolai B, et al: Non-invasive study of *Octopus vulgaris* arm morphology using ultrasound. *J Exp Biol* 214(22):3727–3731, 2011.
47. Martin-Robichaud DJ, Rommens M: Assessment of sex and evaluation of ovarian maturation of fish using ultrasonography. *Aquacult Res* 32(2):113–120, 2001.
48. Mathews KG, Wolff PL, Petrini KR, et al: Ultrasonographic diagnosis and surgical treatment of cystic reproductive tract disease in a female koala (*Phascolarctos cinereus*). *J Zoo Wildl Med* 26(3):440–452, 1995.
49. McQuinn TC, Bratoeva M, DeAlmeida A, et al: High-frequency ultrasonographic imaging of avian cardiovascular development. *Dev Dyn* 236(12):3503–3513, 2007.
50. Melnychuk VL, Cooper MW, Kirby JD, et al: Use of ultrasonography to characterize ovarian status in chicken. *Poult Sci* 81(6):892–895, 2002.
51. Merritt CR: Ultrasound safety: What are the issues? *Radiology* 173(2):304–306, 1989.
52. Morris PJ, Alberts AC: Determination of sex in white-throated monitors (*Varanus albigularis*), Gila monsters (*Heloderma suspectum*), and beaded lizards (*H. horridum*) using two-dimensional ultrasound imaging. *J Zoo Wildl Med* 27(3):371–377, 1996.
53. Mutalib A, Holland M, Barnes HJ, et al: Ultrasound for detecting osteomyelitis in turkeys. *Avian Dis* 40(2):321–325, 1996.
54. Nordberg C, O'Brien RT, Paul-Murphy J, et al: Ultrasound examination and guided fine-needle aspiration of the liver in Amazon parrots (*Amazona* species). *J Avian Med Surg* 14(3):180–184, 2000.
55. Novelo ND, Tiersch TR: A review of the use of ultrasonography in fish reproduction. *N Am J Aquacult* 74(2):169–181, 2012.
56. O'Grady JP, Yeager CH, Thomas W, et al: Practical applications of real time ultrasound scanning to problems of zoo veterinary medicine. *J Zoo Anim Med* 9(2):52–56, 1978.
57. Pees M, Krautwald-Junghanns M-E: Avian echocardiography. *Semin Avian Exotic Pet Med* 14(1):14–21, 2005.
58. Pees M, Kiefer I, Krautwald-Junghanns M-E, et al: Comparative ultrasonographic investigations of the gastrointestinal tract and the liver in healthy and diseased pigeons. *Vet Radiol Ultrasound* 47(4):370–375, 2006.
59. Probert P, Shannon R: Wideband ultrasound to determine lipid concentration in fish. In Halliwell M, Wells PNT, editors: *Acoustical imaging*, New York, 2002, Kluwer Academic Publishers, pp 381–388.
60. Robeck TR, Rostal DC, Burchfield PM, et al: Ultrasound imaging of reproductive organs and eggs in Galapagos tortoises, *Geochelone elephantopus* spp. *Zoo Biol* 9(5):349–359, 1990.
61. Roellig K, Drews B, Goeritz F, et al: The long gestation of the small naked mole-rat (*Heterocephalus glaber* Rüppell, 1842) studied with ultrasound biomicroscopy and 3D-ultrasonography. *PLoS ONE* 6(3):e17744, 2011.
62. Rostal DC, Robeck TR, Owens DW, et al: Ultrasound imaging of ovaries and eggs in Kemp's ridley sea turtles (*Lepidochelys kempi*). *J Zoo Wildl Med* 21(1):27–35, 1990.
63. Royen HIF, Delemarre BJM, Klaver PSJ, et al: Application of transthoracic and transoesophageal echocardiography in captive chimpanzees (*Pan troglodytes*). In *Verhandlungsbericht des 36 internationalen Symposiums über die Erkrankungen der Zootiere*, Kristiansand, Norway, 1994, pp 147–150.
64. Sainsbury AW, Gili C: Ultrasonographic anatomy and scanning technique of the coelomic organs of the Bosc monitor (*Varanus exanthematicus*). *J Zoo Wildl Med* 22(4):421–433, 1991.
65. Sande RD, Poppe TT: Diagnostic ultrasound examination and echocardiography in Atlantic salmon (*Salmo salar*). *Vet Radiol Ultrasound* 36(6): 551–558, 1995.
66. Schroeder K, Drews B, Roellig K, et al: *In vivo* tissue sampling of embryonic resorption sites using ultrasound guided biopsy. *Theriogenology* 76(4):778–784, 2011.
67. Sklansky M, Renner M, Clough P, et al: Fetal echocardiographic evaluation of the bottlenose dolphin (*Tursiops truncatus*). *J Zoo Wildl Med* 41(1):35–43, 2010.
68. Stetter MD, Cook RA: Normal and pathological ultrastrasonographic anatomy of amphibians. In *Proceedings of the American Association of Zoo Veterinarians and Association of Reptilian and Amphibian Veterinarians Joint Conference*, Pittsburgh, PA, 1994, pp 69–70. (abstract).
69. Stetter MD: Noninfectious medical disorders of amphibians. *Semin Avian Exotic Pet Med* 4(1):49–55, 1995.
70. Stouffer JR, Wallentine MV, Wellington GH, et al: Development and application of ultrasonic methods for measuring fat thickness and ribeye area in cattle and hogs. *J Anim Sci* 20:759–767, 1961.
71. Stouffer JR: History of ultrasound in animal science. *J Ultrasound Med* 23(5):577–584, 2004.
72. Wagner WM, Kirberger RM: Transcutaneous ultrasonography of the coelomic viscera of the ostrich (*Struthio camelus*). *Vet Radiol Ultrasound* 42(6):546–552, 2001.
73. Walzer C, Hittmair K, Walzer-Wagner C: Ultrasonographic identification and characterization of splenic nodular lipomatosis or myelolipomas in cheetahs (*Acinonyx jubatus*). *Vet Radiol Ultrasound* 37(4):289–292, 1996.
74. Whittamore JM, Bloomer C, Hanna GM, et al: Evaluating ultrasonography as a non-lethal method for the assessment of maturity in oviparous elasmobranchs. *Marine Biol* 157(12):2613–2624, 2010.