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REVIEW

Advances in ultrasonography and its applications in domestic ruminants and other farm animals reproduction

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Abstract Ultrasound techniques are becoming increasingly important in animal reproduction, offering both a mean of diagnosis and a useful therapeutic tool. Accordingly, understanding the use of ultrasound technology is critical in contemporary animal sciences, since ultrasound examinations are now a routine component of diagnostic workups in reproduction. Ultrasound technology offers the assessment of pregnancy status and foetal viability early post breeding in order to identify animals that fail to conceive, improving reproductive efficiency; early identification of animals carrying twin foetuses, allowing for the implementation of differential management strategies to avoid the negative effects of twinning on general health of the mother animal and also at parturition; and the visualisation of ovarian and uterine pathologies not accurately detected via rectal palpation, allowing appropriate therapies to be implemented. In addition, determination of foetal sex in utero can be done by ultrasonography. The new information that has been generated through ultrasound has thrown light on therapeutic uses, thereby opening up new areas for research. Moreover, ultrasound-guided interventional techniques can be used for diagnostic or therapeutic purposes. In this review, advances and applications of ultrasonography in domestic animal reproduction are reviewed.

Introduction

The application of real-time ultrasonography to study animal reproduction represents a technological breakthrough that has revolutionised knowledge of reproductive biology. New information generated through ultrasonic imaging has clarified the nature of complex reproductive processes in animals, including ovarian follicular dynamics, corpus luteum function, and foetal development.

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Until recently, the techniques used in studying patterns of follicular development involved the measurement, counting and histological evaluation of ovaries of animals killed at various stages during the oestrous cycle, or marking of follicles with ink, followed by serial laparoscopy. In contrast, the development of ultrasonic probes that can be used intrarectally to visualise ovaries has opened up new possibilities for examining the dynamics of follicular growth and regression [1] and provided a means for repeated, direct, non-invasive monitoring and measuring of follicles within the ovary [2]. Early utilisation of ultrasound technology in the dairy industry has included applications such as transvaginal follicular aspiration and oocyte recovery [3], and as a complementary technology for embryo transfer procedures. In addition, early pregnancy diagnosis and monitoring of foetal viability is a great advantage of ultrasonography.

The purpose of this review is to throw light on recent advances and practical applications of ultrasound in animal reproduction.

Development of diagnostic ultrasound

Ultrasound is defined as any sound frequency above the normal hearing range of the human ear; i.e. greater than 20,000 Hz. Sound waves in ultrasound devices are typically produced by vibrations of specialised crystals (piezoelectric crystals) housed in an ultrasound transducer, with the vibrations of the crystals themselves produced by pulses of electric current. A proportion of the sound waves reflected back to the transducer is converted to electric current and displayed as an echo on the ultrasound viewing screen. The transducer, therefore, acts as both the sender and receiver of echoes. The echoes are evident on the viewing screen as varying shades of gray (black to white) [4].

Early applications of ultrasound as a diagnostic aid in medicine utilised Amplitude or A-mode ultrasound. Early applications using A-mode ultrasound included imaging the human abdomen to identify gallstones and foreign material [5], imaging in obstetrics and in the eye [6]. The first use of ultrasound as a diagnostic aid in veterinary medicine was for the detection of pregnancy in sheep [7]. Nowadays, Brightness (B) mode and Doppler are more commonly used than A-mode, and a variety of applications have emerged using these techniques.

The introduction of computer systems to ultrasound machines has enabled the storage, processing and presenting of large amounts of data, allowing the production of static two-dimensional grey scale images and real-time imaging [8]. This real-time B-mode imaging is currently the form of ultrasound most commonly used (Fig. 1).

Prior to real-time imaging, the examination of moving structures such as the heart required a technique now known as Time Motion or M-mode ultrasound. As early as 1954, this form of imaging was used to assess the movement of heart valves and walls [9]. However, neither B- nor M-mode is capable of assessing blood flow.

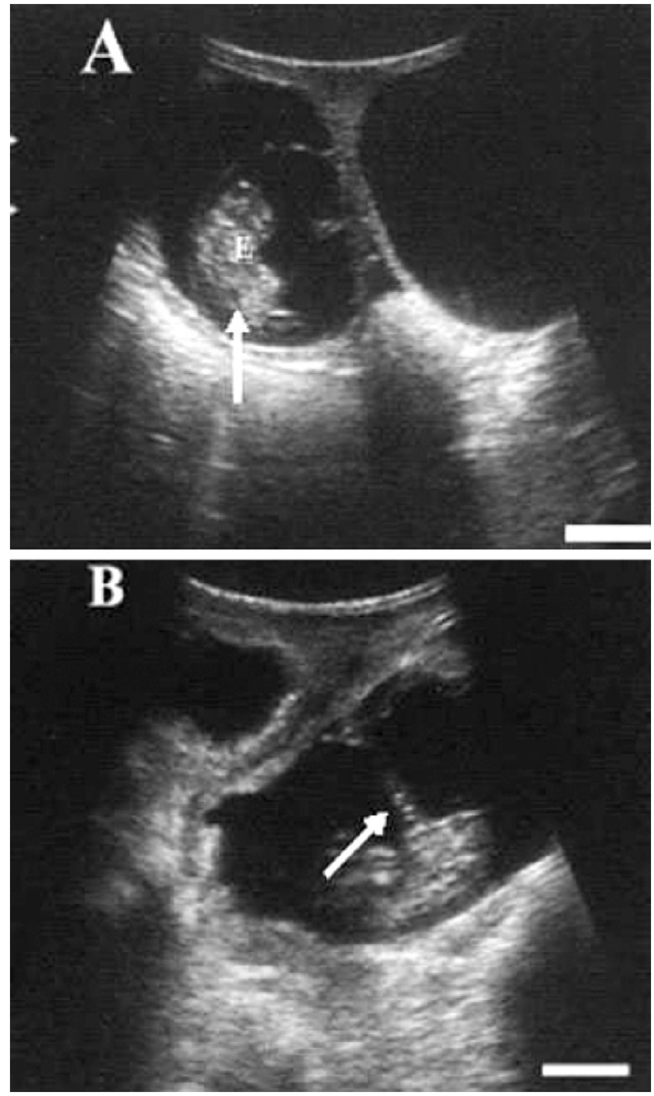

Figure 1 Ultrasound images of goat embryo at day 30 (A) and 35 (B) of gestation. The embryo (E, arrow) observed as an area of high echogenic density. Image B shows the umbilical cord (arrow). Scale bars represent 10 mm [19].

Doppler ultrasound

When an ultrasound beam encounters a moving object such as a red blood cell in vascular flow, the frequency of the returning echo is altered. An increase in frequency occurs when the object is travelling towards the transducer; this is known as positive Doppler shift. An object travelling away results in reduced frequency and a negative Doppler shift. The measurement of these alterations in the returning echo allows the direction and velocity of the flow encountered to be determined [10]. With this, colour flow Doppler is used to screen uterine [11] and testicular [12] blood flow and in the diagnosis of the ovarian cysts in cattle [13].

Recent advances in ultrasound

Advances in hardware and software

Recent advances in diagnostic ultrasound have resulted from concurrent developments in the computer industry and a reduction in the size of component parts, significantly influencing equipment design. Ultrasound machines are currently available in a huge range of sizes. Battery operated hand-carried ultrasound scanners are available [14]. Although initially intended for use in small animals, this type of equipment is now increasingly being used in conservation projects for the reproductive management of farm, wild and captive endangered species including elephants [15,16] and rhinoceros [17]. All of these machines are capable of B and M-mode real-time imaging and many now also incorporate colour flow Doppler, increasing the scope of the examinations that can be performed.

All ultrasound machines allow individual images to be captured and displayed. Combining computer technology with medical ultrasound can help with displaying the data in a more appropriate fashion. With this, advanced post-processing functions have given the operator greater ability to optimise image quality, therefore allowing the production of vastly superior images and Doppler traces. For example, three-dimensional ultrasound has been used in horses to examine the reproductive tract [18]. Moreover, four-dimensional ultrasound, where three-dimensional images are viewed in real-time, is now available.

Techniques of examination

For transrectal or transcutaneous ultrasound scanning in animals, no sedation is required, as the procedure is totally non-invasive and well tolerated. Adequate restraint is required and the scanner should be placed at a sensible distance from the animal on the side opposite the operator's rectalling arm. All precautions that apply to palpation per rectum are applicable to transrectal scanning. All faeces from the rectum should be evacuated prior to introduction of the transducer. It is often advantageous to carry out a preliminary exploration of the topography of the reproductive tract before commencing the ultrasonographic examination. The transducer face must be lubricated with a suitable coupling medium and is usually covered with a lubricated plastic sleeve before insertion in a cupped, lubricated hand through the anal opening in large animals or by using a rod stick in small animals [19]. It is then progressed cranially along the rectal floor to overlie the reproductive tract. The transducer face must be pressed firmly against the rectal mucosa in order to effect ultrasound transmission through the rectal wall into the abdominal viscera. The probe is moved across the reproductive tract in a thorough and systemic manner.

Applications of ultrasound in domestic animal reproduction

The ability of ultrasound to distinguish fluid from soft tissue and differentiate between soft tissues based on their composition makes it better than radiography for examining soft tissue structures [20]. Ultrasound therefore provides a non-invasive alternative to many radiographic contrast procedures, though the two techniques should still be considered as complimentary. Ultrasound may also often provide information that was previously only available through exploratory laparotomy. Further applications of ultrasound include identifying pregnancy and foetal number determination [21]. Ultrasound also permits foetal sexing [22].

As a pregnancy diagnosis method, transrectal ultrasonography is accurate and rapid, and the outcome of the test is known immediately at the time the test is conducted. The rate of embryonic mortality and the efficacy of strategies to rebreed cows at various stages post breeding also play a role in determining the advantages and disadvantages of the timing of pregnancy diagnosis and resynchronisation [23].

Assessment of normal ovarian structures

Follicles

The ultrasonographic anatomy of the ovaries of the cow has been described in detail. Antral follicles of various sizes appear as non-echogenic structures, which can be distinguished from blood vessels in cross-section by the elongated appearance of the latter [24]. A linear relationship has been shown between follicle diameter measured by *in vivo* ultrasonography and follicle diameter determined after slaughter [25]. Correlation coefficients of 0.7–0.9 for various sizes of follicular structures have been recorded between *in vivo* ultrasonography and post-mortem slicing of excised ovaries [26]. In goats, [27] have reported that transrectal ultrasonography is a reliable method for studying follicular dynamics.

Ovulation

Determination of ovulation by ultrasound examination has been reported [28]. In this, the ovaries of 8 heifers were examined in one investigation by ultrasonography every 4th hour during and after oestrus. Ovulation was depicted by the absence of a preovulatory follicle that was present at a previous examination and subsequently confirmed by the development of corpus luteum at the same spot. The usefulness of ultrasonography performed at 2-hourly intervals for detecting the onset of ovulation has also been demonstrated [29].

Corpora lutea

The ultrasonic characteristics of corpora lutea (CL) have been described [30]. Generally, a CL is identified ultrasonically from the third day after ovulation. A developing CL appears on the ultrasound image as a poorly defined, irregular, greyish-black structure with echogenic spots all within the ovary; a mid-cycle CL is a well defined granular, greyish echogenic structure with a demarcation line visible between it and the ovarian stroma; in a regressing CL the demarcation line is faint, owing to the slight difference in echogenicity between the tissues [31].

In small ruminants such as goats, where we cannot examine ovarian structure through palpation per rectum, ultrasound is the best method for monitoring ovarian activity as mentioned [27].

Pregnancy diagnosis

Early pregnancy diagnosis can improve reproductive performance by decreasing the interval between successive artificial insemination services and coupling a non-pregnancy diagnosis with an aggressive strategy to rapidly rebreed the animal [32].

Pregnancy diagnosis in cattle can be achieved by ultrasonography. In this the foetus appears as an echogenic structure inside a non-echogenic structure [33]. To compensate for embryonic mortality, cows diagnosed pregnant early post breeding must undergo one or more subsequent pregnancy examinations to identify and rebreed cows that experience embryonic mortality. This applies to all methods for early pregnancy diagnosis including transrectal palpation conducted before the rate of embryonic mortality decreases. Thus, dairy managers who have implemented early pregnancy diagnoses must consider the timing and frequency of subsequent pregnancy examinations to maintain the reproductive performance of the herd.

Determination of foetal number and viability

The ability to identify multiple foetuses with real-time ultrasonography is a clear advantage over other techniques. In a previous study [19]; Table 1, foetal number in goats was shown as detectable at day 40 post-mating; the best time was day 60 after mating. Determination of foetal number would allow producers to separate animals carrying singles, twins or triplets for differential management. Fig. 2 shows foetal number as detected by ultrasound. Cows carrying twin foetuses can be accurately identified using transrectal ultrasonography by 40–55 days post artificial insemination [34]. Determination of foetal viability is a clear advantage of ultrasound over other methods of pregnancy diagnosis. The heart contractility can be seen between the ribs (Fig. 3) during examination.

Foetal sex determination by ultrasonography

Foetal sex determination has several implications in the animal breeding industry. The gender of foetuses can be detected by visualisation of the location of the genital tubercle [35] or the scrotum and mammary glands [36]. The most appropriate time of ultrasonographic sex determination is 55–60 days of gestation and the technique can be accurate even under farm conditions [37]. Foetuses at 48–119 days of age have been successfully sexed [38]. The procedure is reliable and the accuracy has ranged from 92 to 100%

Table 1 Accuracy of early pregnancy diagnosis in goats by plasma progesterone assay or real-time B-mode ultrasonography and detection of foetal number [19].

	No.	No. correct[a]	Accuracy (%)
Progesterone assay[b]			
Pregnant	15	12	12/15 (80)
Non-pregnant	3	3	3/3 (100)
Ultrasonography[c]			
Pregnant	12	12	12/12 (100)
Non-pregnant	6	6	6/6 (100)
Foetal number[d]			
Day 40 after mating	12	8	8/12 (66.7)
Day 50 after mating	12	10	10/12 (83.3)
Day 60 after mating	12	11	11/12 (91.7)

[a] Determined at kidding or caesarean section.
[b] Measured at day 21 after oestrus and the cut-off value used was 1 ng/ml.
[c] Confirmed by the detection of embryo and its heartbeats at 24.3 ± 0.7 days of gestation.
[d] Diagnosed by ultrasonography by finding more than one heart, skull and sets of ribs.

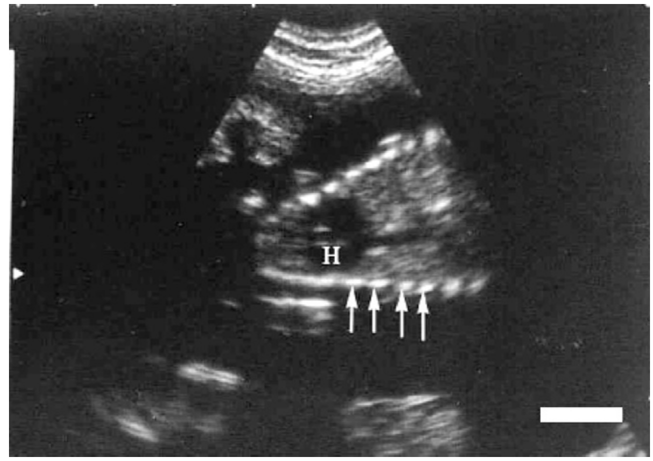

Figure 3 Ultrasound image of the thorax in a goat foetus at 2 months of gestation obtained using a 5 MHz transabdominal transducer (note that the heart (H) appears as an anechoic structure between the white dots which represent ribs, arrows). Scale bar represents 10 mm [19].

[36]. Beal et al. [38] have noted that 84 out of 85 foetuses predicted to be male were confirmed correct, resulting in 99% accuracy.

In production dairy systems, determination of foetal sex is useful when combined with a management decision or strategy that justifies the expense of foetal sexing [32]. In other words, a dairy producer who pays for information regarding foetal sex must economically justify the usefulness of that information. Fulfilling sales contract obligations regarding the sex of a calf carried by a pregnant cow to be sold is one scenario that may justify this expense. It should be emphasised however, that ultrasonic identification of the genital tubercle or the scrotum and mammary glands for sexing purposes requires considerable experience.

Determination of foetal age

Estimation of foetal age, monitoring of foetal growth across time and diagnosis of pregnancy disorders can be performed by ultrasonographic foetometry. Biparietal diameter of the skull and length of the long bones, as seen in Fig. 4, can be used to estimate gestational age. Growth curves of foetal structures based on ultrasonographic foetometry have been reported [39]. In this, the sonographic foetometry of foetuses in 19 pregnant heifers have been described in detail. A total of 485 examinations were carried out from 2 to 10 months of pregnancy. The organs evaluated included eyeball, metacarpal diaphysis, os ilium and os ischii and scrotum. Ultrasonographic foetometry has been shown to provide a precise estimation of gestational age and prediction of calving dates [40]. This investigation concluded with the assertion that the accuracy and precision of the prediction of calving date were sufficient to be of benefit in the management of cows in late pregnancy and at calving.

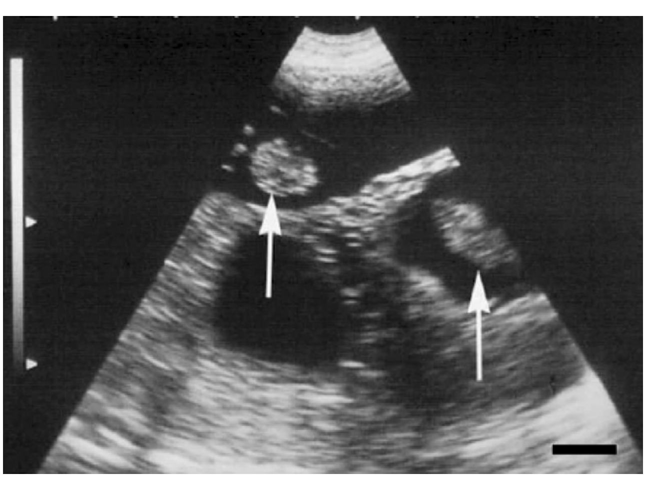

Figure 2 Ultrasound image of a twin in goats (arrows) at day 40 of gestation. Scale bar represents 10 mm [19].

Interventional techniques

Ultrasound-guided transvaginal oocyte aspiration is helpful in obtaining ova from clinically infertile but otherwise valuable cows for *in vitro* fertilisation. In this way, the genetic potential of such donor cows can be propagated.

Guided needle placement

All types of transducer can be used to guide needle placement [41]. The needle can be directed through a channel in the transducer itself, via an attachable biopsy guide or by free hand. When passed across the beam, the shaft and tip are clearly visible allowing the path of the needle to be determined and precise placement of the tip for the removal of material or the introduction of a diagnostic or therapeutic agent. Ultrasound-guided interventional techniques are used commercially in cattle to facilitate follicular aspiration and embryo transfer [42]. This technology has also been applied in mares [43], goats [44] and buffalo [45]. Routinely performed diagnostic sampling techniques, including fluid aspiration, fine needle aspirates and core biopsies are common components of clinical diagnostic workups in many species [20].

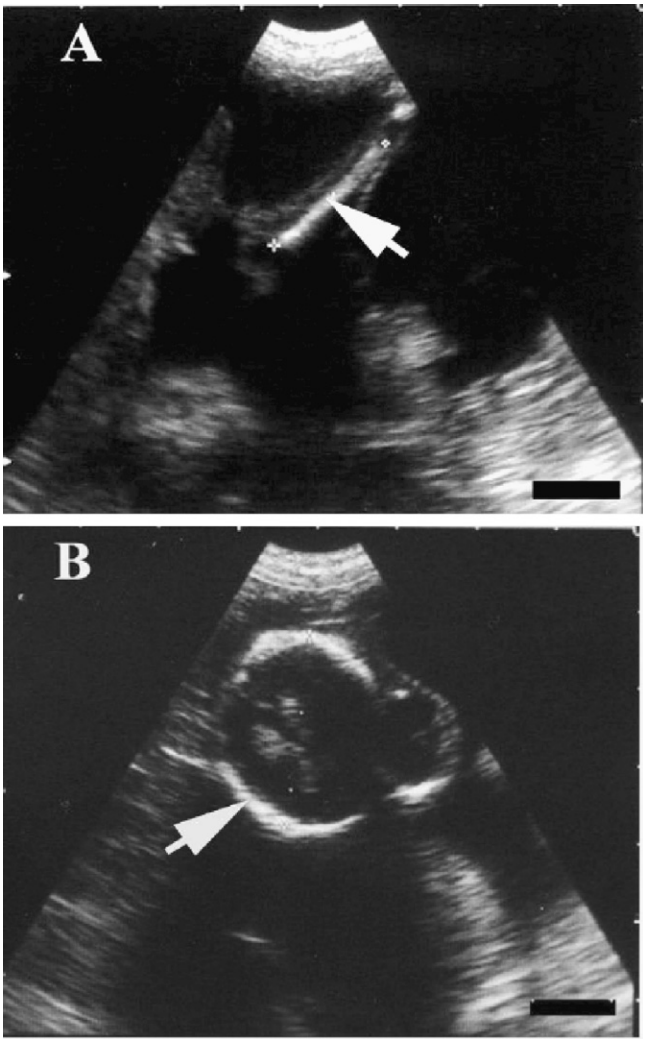

Figure 4 Ultrasound image of a long bone (radius; arrow A) and the head (arrow B) of a goat foetus on day 70 of gestation obtained using a 5 MHz transabdominal transducer. Scale bars represent 10 mm [19].

Therapeutic ultrasound

In a trial for using ultrasound for therapeutic purpose, Sasaki and his coworkers [46] fabricated a prototype 3.25-MHz split-focus therapeutic transducer combined with a small 6.5-MHz imaging ultrasonic probe for transrectal treatment of prostate cancer, evaluating the feasibility of using split-focus high-intensity focused ultrasound (HIFU) to ablate localised tumour tissue without injuring the surrounding organs. They established a localised tumour model by inoculating VX2 tumour into rabbit livers. The localised VX2 tumours of nine rabbits were transdermally treated with split-focus ablation at a peak intensity in water of 6 kW/cm^2 for 4 s (6 shots) under the guidance of ultrasonic B-mode imaging. Necropsy a day after treatment found the surface of the livers and gastrointestinal tracts to be grossly normal. The VX2 tumours were completely coagulated and were surrounded by ablated liver tissue. The six shots of split-focus HIFU destroyed the VX2 tumours without injuring the liver surfaces or the surrounding organs. These results suggest that split-focus HIFU ablation could be an effective treatment of localised tumours.

How safe is diagnostic ultrasonography?

As discussed above, the benefits of ultrasound as a diagnostic imaging procedure in animal reproduction are numerous. Importantly, routine examinations have been shown to have no harmful biological effects. Ultrasound is considered a safe procedure for the animal, the operator and nearby personnel, allowing it to be performed in any location without the need for specific safety precautions. It is non-invasive and therefore well tolerated in animals, making serial examinations, such as to monitor progression of the condition, response to treatment or to practice scanning techniques, possible [20].

Ultrasound is a wave form of non-ionising energy. It has no relation to X-rays, which damage tissues because of their ionising effect on living cells. The low intensity of pulsed ultrasound used for diagnostic purposes and in the Doppler devices designed for foetal monitoring produces no significant heating. However, other Doppler devices do use intensities that may produce significant heating and are not suitable for foetal monitoring. Another bioeffect of ultrasound is cavitation, which is a complex phenomenon in which gas-filled bubbles enlarge in an ultrasound field. At high intensities these bubbles may collapse suddenly, causing large but localised increases in temperature, thermal decomposition of water and release of free radicals. This phenomenon has been termed transient cavitation [47,48]. Diagnostic ultrasound contrast agents have been developed for enhancing the echogenicity [49]. However, bioeffects of contrast-aided diagnostic ultrasound happen on a microscopic scale and their importance in the clinical setting needs more investigation [50].

Compared with other diagnostic aids as X-rays, ultrasound is considered very safe, with no harmful bioeffects. However, the question of long-term biologic effects of diagnostic levels of ultrasound cannot yet be answered and require more investigation.

Conclusion

The impact of real-time ultrasound on the study of animal reproduction has been dramatic, and development of portable ultrasound machines has given clinicians an added tool for diagnostic reproductive management. Ultrasound is commonly used to monitor uterine anatomy, involution and pathology. In addition, it has been used to detect pregnancy, study embryonic mortality, monitor foetal development, and determine foetal sex. Recent advances in ultrasound technology in both hardware and software have resulted in the production of superior images and the widespread use of ultrasound.

Compared with other diagnostic aids such as X-rays, ultrasound is considered very safe and has no harmful bioeffects. Another advantage of ultrasound is its real-time nature in examination, allowing studies of moving structures.

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