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Basics of ultrasonographic examination in sheep

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ABSTRACT

Diagnostic ultrasound has become an essential tool in veterinary medicine and is well established in a variety of species. Since its introduction into sheep clinical practice, it has been mainly used in pregnancy diagnosis and identification of litter size, as well as in experimental settings in reproductive medicine. Furthermore, sheep frequently serve as an experimental model in several disciplines and sub-disciplines of human medicine. However, despite of its great potential, ultrasonography does not yet play a major role in general clinical practice. The review focuses on ultrasonography in sheep and provides information in basics of ultrasound imaging. These include physical principles, instrumentation, handling and preparation of animals, imaging modes (amplitude-depth mode, brightness mode, time-motion mode, 3- and 4-dimensional ultrasound, Doppler ultrasonography), and interpretation of ultrasound images and artefacts. Examples of clinical and experimental applications are included with regard to the different imaging modes, to demonstrate how ultrasonography can be used in sheep clinical practice and research. Additionally, animal welfare as well as safety and hygiene aspects are considered.

1. Introduction

Ultrasound has a frequency greater than 20 kHz, which is above the range of human hearing. It allows communication, food acquisition or orientation in bats and other animals, but can also be used as a technique of diagnostic medical two- and three-dimensional or flow imaging. Meanwhile, ultrasonography (from Latin words '*ultra*': more and '*sono*': sound and Greek word 'graphein' ['*γράφειν*']: to write) or ultrasound imaging is one of the most widely used powerful tools in animal diagnostics and research, which allows the real-time visualisation of internal organs, tissues, structures or fluid accumulations and the investigation of blood flow without any radiation risk. It depends on the computerised analysis of reflected ultrasound waves. Due to its non-invasive character and apparent high safety, it has become one of the most rapidly advancing technologies in the clinical fields.

Although sheep have frequently been used as animal models for humans, ultrasonography in sheep veterinary practice has not yet received the same attention as in other domestic species, except pregnancy diagnosis and identification of litter size in ewes. Presumably, this seems to be the consequence of financial considerations. Depending on breed, value of individual animals is often quite low, hence owners may be reluctant to invest a larger amount of money. Nevertheless, as nowadays many animal practitioners possess ultrasonography equipment suitable for a variety of species, its use for clinical diagnostics and follow-up studies is also rising in sheep. Further, increasing interest in animal welfare issues might hopefully influence positively willingness to spend increased money for extended diagnostics in this species.

Ultrasonography belongs to 'animal-friendly' techniques; particularly when transcutaneous ultrasonographic investigations are performed, these are associated with only minor stress for all animals, which are habituated to competent handling during routine husbandry. However, this depends in part on the individual temperament of the sheep. It should be kept in mind that animals can be trained to voluntarily accept restraint (Grandin, 1989). If required, ultrasonographic investigations can also be performed transrectally, transvaginally, transoesophageally or intraoperatively/endoluminally. This may, in these cases, necessitate sedation and local or general anaesthesia.

Since standard ultrasonic images offer a cross-sectional view of structures, tissues and organs which can be acquired in 'real time', they can provide visual guidance for many medical interventions, too. These include tissue biopsies or ablations, oocyte collection and embryo transfer, or regional anaesthesia, to name only some of them. Correct image interpretation requires profound knowledge of animal anatomy and physiology, as well as of the abilities of gas, fluids, tissues and organs to reflect sound waves.

The present review for veterinarians and researchers active in the field of sheep biology and health management includes information in safety, hygiene and welfare aspects, as well as in

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principles, methods and potential applications of various ultrasonographic medical techniques. It is based on clinical and experimental data in sheep, but also on literature with regard to other species including humans.

2. General considerations

2.1. Safety

According to the present knowledge, ultrasound is considered to be a safe technique (for review see e.g. Miller, 2008). Anyhow, Aiken and Lees (2012) recommended continuing to be prudent, especially when exposing embryos and first-trimestre fetuses to Doppler ultrasound, because of possible long-term developmental implications. Further, it has been stated by the European Commitee for Medical Ultrasound Safety (2015): "Ultrasound produces heating, pressure changes and mechanical disturbances in tissue. Diagnostic levels of ultrasound are capable of producing temperature rises that may be hazardous to sensitive organs and the embryo/fetus. Biological effects of non-thermal origin have been reported in animals". Since spectral pulsed Doppler mode and colour Doppler imaging can produce temperature rises, diagnostic ultrasound in early pregnancy should be prudently used (British Medical Ultrasound Society, 2012). Recommendations on exposure times and index values for obstetric ultrasound in humans (Safety Group of the British Medical Ultrasound Society, 2009) may serve as reference for veterinary purposes.

2.2. Equipment, facilities, handling, animal welfare

Ultrasound equipment used for clinical investigations in sheep in the field should be small enough to be easily portable, even under extreme conditions, e.g. in high mountain regions, and preferably independent from public electricity network for prolonged time periods. Meanwhile, storage battery driven, hand-held and adapter or wireless connected equipment is available on the market; probes (transducers) can be used with a notebook, tablet or smartphone equipped with special software. In contrast, larger transportable ultrasound units might be preferred in farms, whereas special conditions in clinics allow the use of large-sized, technically superior non-transportable machines, although miniaturisation is the trend.

During outdoor examinations in the field, ultrasound equipment often has to be protected from humidity or temperature extremes. This can be done by placing the ultrasound apparatus and other equipment in suitable hard-cases lined with polystyrene sheets and supplemented with moisture absorbers, as well as cooling/heating gel pads. Light or light reflection might impair the view of the images. In these cases, a tent or a small tent-like construction prepared with an insulation blanket or a beach shelter often can provide enough shade for a proper view.

Especially in clinics, facilities suitable for ultrasonography are available at all times. Rooms should be inviting, appropriate for being darkened and should be equipped with well-designed and easily-operated handling systems or other devices (e.g., special gates, stocks, chutes, adjustable fitting stands, turning cradles, tilt tables, operating room tables). These systems/devices should allow a comfortable but proper restraint of animals, preferably in the standing position or otherwise restrained on their side or in sternal or dorsal recumbency. In farms or in the field, improvisation might become necessary; examination can be performed in the milking parlour, with animals restrained against a wall or fence, sitting or with the help of portable handling systems or in any other useful way.

Facilities for proper restraint of animals are particularly necessary when the ultrasound probe is introduced intrarectally, intravaginally or intraoesophageally, to prevent any trauma and to perform a safe and thorough examination. For welfare reasons, but in dependence on the purpose of the intervention, sheep in dorsal recumbency should not be presented with hyperextension of head and neck or completely outstretched limbs. Instead, a natural head posture and a comfortable flexion of the large joints of the limbs should be chosen. Cushions should be provided for leg restraint and in emaciated individuals also for the spine. Restraining systems should allow presentation of the animals at a comfortable working height, as well as with elevated head or hindquarters.

Sheep are social animals and feel distressed and threatened when separated from their flock (Canadian Sheep Federation and the National Farm Animal Care Council, 2013). Therefore, they should be examined, if possible, in close proximity of a group of familiar flock mates and in a quiet environment. If facilities only allow presence of single animals, wallpapers or portrait photos of familiar sheep make the situation less stressful. Although it is often not possible in cases of clinical illness, all routine and serial ultrasonic examinations should be accompanied with arrangements of positive enhancement (e.g. feeding).

2.3. Preparation of animals

Transcutaneous scanning can be done after shearing/shaving the area above the region of interest or after parting of wool or hair to create an observation window. The latter should be preferred in the case of severe weather conditions or in sheep which will be presented e.g. in shows. Especially for pregnancy diagnosis, also the fleece-less area adjacent to the udder can be used for scanning without clipping. In order to create proper contact for the transducer, a thorough cleaning of the skin might be helpful. Shearing/shaving should be performed in every case of intraoperative scanning and in other cases of higher infection risk, when the skin is perforated and/or body/organ cavities are reached during ultrasonically controlled manipulations (e.g., shearing of the perineal area, tail and tail base before transvaginal aspiration of ovarian follicle contents for oocyte collection). Further, before the interventions, the clipped area should be thoroughly washed with an antibacterial soap or washing lotion and rinsed with 70% ethanol or, depending on location, treated carefully with a suitable mucosa disinfection solution.

Ultrasonic coupling media facilitate transmission of sound energy between transducer and tissue and reduce attenuating effects. Their use is necessary, because ultrasound energy is not effectively transmitted through air. Self-made gels (recipes available in the internet, e.g., Lutz, 2011), vegetable oil (Watt et al., 1984) or alcohol (e.g., 70% isopropyl alcohol; Jones et al., 2016) have been reported as alternatives for commercial coupling gels for transcutaneous scanning. Dependent on the ultrasonic approach and the infection risk, coupling gels should be sterilised. For transrectal examinations, lubricant gel is introduced into the rectum. A preceding removal of faeces is mostly dispensable and can be considered as unnecessary stress for the animals. Lubricant gel for transvaginal investigations should be tested for tissue and sperm compatibility, since it has been reported that those gels can be toxic for spermatozoa (Vargas et al., 2011); in that case, pre-warmed, sterile physiological saline is normally already sufficient for lubrication. Sterile saline can also be used intraoperatively as transmission medium.

2.4. Hygiene

Ultrasound transducers, especially those used for intraoperative/intracavitary scanning, should be covered with convenient and if necessary sterile material, e.g., plastic sleeves, surgical gloves or condoms (without spermicides: obligatory when used intrav-

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