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Review

ULTRASONOGRAPHY OF THE UDDER AND TEAT IN CATTLE: PERSPECTIVE MEASURING TECHNIQUE

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ABSTRACT

The usage of modern, accurate and relatively quick methods for udder examination like ultrasonography are necessary for regard udder illnesses and health problems, due to their negative impact on the milk production and economics of herds. The aim of this review is to call attention to the possibility of the use of ultrasonography as a useful tool for the evaluation of morphological characteristics of the udder and teats in dairy cattle, to summarize the data related to ultrasound examination in cows, to show usable scanning methods and the interpretation of the ultrasonographic image for better understanding of this method. Ultrasonography is a noninvasive technique that can be used for examining the bovine udder and teats. It can be used as a helpful tool to diagnose pathological alterations in the udder such as inflammation, mucosal lesions, tissue proliferation, foreign bodies, milk stones, congenital changes, hematoma and abscess. Udder and teat scanning can be also performed for diagnosis of milk flow disturbances and different inner anatomical structures of the teat like teat canal length and diameter, teat cistern diameter, and teat wall thickness. The ultrasound examination of the udder parenchyma is mainly performed using the direct contact method with lower frequency linear probes (3.5 – 5 MHz). Examination of the teat is most commonly conducted by the water bath technique with a help of a higher frequency linear probes (at least 7.5 MHz) for better image quality.

Key words: ultrasonography; ultrasound; udder; teat

INTRODUCTION

The quantity and quality of produced milk as well as the efficiency of its production is directly dependent on good health of cows and in particular cow's udder. Preparation of the mammary gland, milking, technology of milking and care of the udder presents a set of technical operations which significantly affect not only a quality and quantity of milk, but also the economics of a herd. The introduction of mechanized milking and new technologies, although hygiene have improved milk production, on the other hand, became the cause of many failures and changes in efferent teats tract (Twardon *et al*., 2001). From a breeding point of view it is important to keep cows with higher milk production and with well-structured udder and teats, more resistant to long-term impact of milking equipment. For these reasons it is important to breed high-yielding breeds with regularly shaped, well developed udder and teats suitable and more resistant for machine milking (Gere *et. al*., 1999). Optimal conditions in practical dairy farming should be respected and the biological requirements of dairy cows should be followed to maintain high production in combination with animal's good health (Tančin & Bruckmaier, 2001).

The disturbances causing a drop in milk production are a major problem at dairy farms. Different types of mastitis leading to loss and adverse changes

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in the quality of milk, as well as increased costs for treatment and early culling of the animals make up the negative economic effect (Anderson *et al*., 2002; Bergonier *et al*., 2003; Khan & Khan, 2006; Blowey & Edmondson, 2010).

For these reasons an accurate, fast and effective measuring and diagnostic techniques were developed. One of the most effective and most widely used methods worldwide is ultrasonography.

What is ultrasonography?

Real-time ultrasonography has gained tremendous popularity in recent years as a diagnostic as well as a research tool in veterinary and animal science. Reports on the diverse applications of ultrasonography, continued improvements to imaging quality, availability of portable ultrasound scanners, and reduction in equipment costs have led to its general acceptance by the veterinary profession. As a diagnostic aid, ultrasonography is well suited for bovine or ruminant practice, particularly for the examination of reproductive organs (Dimitrov *et al*., 2002; Kahn, 2004; Yotov, 2005). Also it can be used like a helpful tool to diagnose pathological alterations of the udder such as inflammation, mucosal lesions, tissue proliferation, foreign bodies, milk stones, congenital changes, hematoma and abscess etc. The technique is noninvasive, relatively simple and effective, safe to both the subject and the operator, portable, and ultrarapid, since the ultrasonic image facilitates immediate interpretation and diagnosis in most circumstances (Rajama-hendran *et al.*,1994).

Most ultrasound machines consist of a console unit that contains the electronics, controls, and a screen upon which the ultrasound image is visualized by the operator, and a probe (transducer), which emits and receives high-frequency ultrasound waves. Linear-array transducers consist of a series of piezo-electric crystals arranged in a row. These crystals emit high frequency sound waves upon being energized. The configuration of a linear-array transducer results in a rectangular image on the field of scan (as opposed to a pie shaped image produced by a sector transducer). Ultrasound imaging uses high frequency pulses of sound waves (typically 2.5 – 10 MHz for general use) (Fricke, 2002).

Linear-array transducers of 5.0 MHz, 7.5 MHz and 10 MHz frequency ranges are most commonly used in cattle to perform ultrasound examinations, and most veterinary ultrasound scanners are compatible with probes of different frequencies. The depth of tissue penetration of sound waves and image resolution is dependent upon and inversely related to the frequency of the transducer. Thus, a 5-MHz probe results in greater depth of tissue penetration and lesser image detail, whereas a 7.5-MHz probe results in lesser depth of tissue penetration and greater image detail. An ultrasound scanner equipped with a 5-MHz probe is most useful for bovine practitioners conducting routine reproductive examinations; however, the udder structures are best imaged with a 7.5-MHz probe or higher (Fricke, 2002 ; Pock, 2011; Wojtowski *et al.*, 2011).

History of ultrasonography

As for high frequency "ultrasound", Lazzaro Spallanzani, an Italian biologist, could be credited for it's discovery when he demonstrated in 1794 the ability of bats navigating accurately in the dark was through echo reflection from high frequency inaudible sound. The real breakthrough in the evolution of high frequency echo-sounding techniques came when the piezo-electric effect in certain crystals was discovered by Pierre Curie and his brother Jacques Curie in Paris, France in 1880. This finding made possible the generation and reception of ‚ultrasound' that are in the frequency range of millions of cycles per second (megahertz) which could be employed in echo sounding devices. Further research and development in piezo-electricity soon followed (Woo, 2001). In mean time, another scientist Johann Christian Doppler (1803-1853) Austria, called the hypothesis that „ the pitch of a sound would change if the source of the sound was moving".The Color Doppler ultrasound is an important tool in ultrasonography of today.

The next development of ultrasound was made through navy. Sinking of the Titanic 1912 was the impulse for the development of echolocating device for nautical purpose. The first working echo–device called SONAR (sound navigation and ranging) was made in 1914 (Kane *et al*., 2004). During the world wars the use of ultrasound technology was refined and used in Sonar's, Radars or Flaw deflectors. Each of these devices were in their unique ways a precursors of all ultrasonic equipments. The modern ultrasound scanner embraces the concepts and science of all these modalities (Woo, 2001).

Ultrasonography like a safe and effective form of imaging has been used for more than half a century to aid in medicine diagnosis and guide procedures. In veterinary, the ultrasound was originally investigated as a tool for early pregnancy diagnosis, ovarian structures, fetal sexing, fetal aging, etc. (Beal *et al*., 1992; Jones & Beal, 2003; Ribadu and Nakao, 1999). Initially, the high cost of the ultrasound machines dissuaded the use, but over the past two decades, ultrasound equipment has become more compact, higher quality, and less expensive.

The first ultrasonographic examinations of mammary gland as whole were carried out by Caruolo & Mochrie (1967) as an A-mode sonography with a frequency of 1 MHz. Cartee *et al*. (1986) tested B-mode (real-time mode) ultrasonography to diagnose milk flow disturbances (Klein *et al*., 2005). Later, teat examinations were made using frequency about 3.5 and 5.0 MHz (Jenninger, 1989; Stocker *et al*., 1989;

Will *et al.* 1990; Saratsis & Grunert, 1993; Seeh *et al*., 1996). The early integration of ultrasound technology to the dairy industry in 1990's included also such applications as transvaginal follicular aspiration and oocyte recovery (Pieterse *et al*., 1988; Pieterse *et al*., 1991; Meintjes *et al*., 1993) as a complementary technology for embryo transfer procedures.

Udder and teat measuring techniques before ultrasonografy

Twenty years ago, the only way to diagnostic milk secretion anomalies was with help of radiography (Witzig & Hugelshofer, 1984; Witzig *et al*., 1984; Alacam *et al*., 1990). In more recent times this method was replaced by ultrasonography. In support of this statement, Stocker & Rusch (1997) and Hospes & Seeh (1999) pointed out the method's non-invasiveness and the possibility to observe the separate mammary gland structures (teat and parenchyma) without using ionised radiation as its main advantages. Unlike ultrasonography, theloscopy allows only the visualisation of the papillary part of the milk cistern and the teat canal, while the anatomical structures located proximally to them are not displayed (Geishauser *et al*., 2005; Kiossis *et al*., 2009; Fasulkov, 2011).

Also various methods have been described to measure directly the teat tissue. Radiography techniques have been used for many years (Pier *et al*., 1956; McDonald, 1975; Mein *et al*., 1973) and have provided good results. Impedance measurement has been used to measure teat-end congestion indirectly by measuring the flow of blood through teat tissue (Mayntz & Almgren, 1985). Another indirect method to monitor teat tissue reaction via blood flow is the use of infrared thermography to measure the temperature of teat tissue. Skin temperature is used as a parameter that relates to the intensity of the circulation of blood (Hamann & Duck, 1984, Paulrud *et al*., 2005, Kunc *et al*., 2007). The cutimeter, is described as a simple, noninvasive tool for the measurement of teat thickness (Hamann & Mein, 1988). The cutimeter should be used with care, since results are dependent on the time and application of pressure on the teat. Moreover, since pressure is applied to the teats, this method may not be representative of recovery results of teats after milking (Neijenhuis *et al*., 2001). Because of its noninvasive nature, ultrasound seems to be one of the best, direct method to monitor the internal structures of teats.

Methods of ultrasonographic examination

The internal structure of the mammary gland and teats can be studied by means of ultrasonography. Studies using ultrasound equipment in teat diagnostics have been carried out for only a few years now and they generally focus on the development of a methodology to obtain an image of intramammary structures (Franz *et al.*, 2001). Udder and teat scanning is generally performed for diagnosis of milk flow disturbances but also is increasingly used for examination and measurement of different anatomical structures (Ayadi *et al*., 2003; Weiss *et al*., 2004; Paulrud *et al*., 2005).

A number of authors have reported about udder and teat ultrasonography in cows (Will *et al*., 1990; Celik *et al.*, 2008; Sekere *et al*., 2009; Porcionato *et al*., 2010; Nishimura *et al*., 2010 ; Fasulkov, 2012), sheep (Ruberte *et al*., 1994; Rovai *et al*., 2008) and goats (Wojtowski *et al.*, 2002; Fasulkov *et al*., 2010; Ślosarz *et al*., 2010).

The use of a proper most common B-mode ultrasonography equipment allows the differentiation of morphological structures, such as mammary gland parenchyma, gland and teat cisterns, teat wall, rosette of Furstenberg, and teat canal (Franz *et al*., 2001). For this purpose, convex, linear and sector probes should be used (Flock & Winter, 2006; Wojtowski *et al*., 2006; Fasulkov, 2011). Ruberte *et al.* (1994) and Franz *et al.* (2003) used the method to visualize the parenchymal tissue, the mammary canals, the gland and teat cisterns, and the teat wall in sheep. With a help of a B-mode ultrasonic device, Bruckmaier & Blum (1992) were able to observe the changes in the volume of the milk cistern of cows, goats and sheep after treatment with oxytocin.

For ultrasonographic visualization of the structures of the bovine teat, liner probes with a frequency of 3.5-10 MHz are used (Hospes and Seeh, 1999). The ultrasonographic examination between the mammary gland and teat cistern space and mammary gland parenchyma is done mostly through the direct contact method with 5 MHz or higher frequency (7.5 MHz) probe (Cartee *et al*.,1986)

After surface preparing what includes hair shaving, disinfection and contact gel application, the probe is placed on the caudal and lateral surface, directly on the udder skin (Franz *et al.*, 2009). During ultrasonographic scanning, the probe is placed transversely and longitudinally to the udder, designated as vertical and horizontal position respectively (Franz *et al*., 2003; Gungor *et al*., 2005). The direct contact method, can be also used in teat scanning, when the surface of the linear probe is placed directly on the skin surface of the teat. Bruckmaier and Blum (1992) suggested that the presence of air between the probe and the tissue examined must be avoided during ultrasound examination. The problem was usually solved by using contact gel and applying the probe directly to the animal's body. Pressing the ultrasound probe to the tissue leads to teat image deformities. As the connection between the teat and gland is angular, the application of the probe should simultaneously exclude air and prevent teat image deformations as reported by (Franz *et al*., 2001; Twardon *et al*., 2001; Santos *et al*., 2004).

Because of the mentioned risk of deformation, the primary method for ultrasonography of the teat structures is the „water bath „ technique (Stocker *et al*., 1989; Sendağ & Dinc, 1999; Franz *et al*., 2009). It is done by immersing the teat into a plastic bag or cup fulfilled with hot water (30-37 ˚C), with the probe placed on the cup, or build into the cup. The teat can be examined horizontally by placing the probe at a right angle to the axis of the teat, or in a vertical position with the probe parallel to the teat. Cartee *et al*. (1986) reported that the use of the water bath in scans of the mammary glands was to increase the acoustic impedance difference between the teat wall and the surrounding medium. The presence of milk in the teat sinus acted similarly as a window of acoustic impedance for imaging the deeper structures and far wall of the teat. Seeh *et al.* (1996) obtained an image of satisfactory quality through direct contact with the teat with a 3.5 or 5 MHz probes, whereas Stocker *et al*. (1989), Will *et al.* (1990) and Saratsis & Grunert (1993) achieved better visualization using the water-bath technique. Fasulkov *et al.* (2010) reported that transcutaneous teat sonography (direct contact) with a use of a linear 5 MHz probe in goats, did not produce satisfactory results. For better quality of ultrasonographic teat images, most of the authors recommend using of frequency no less than 7.5 MHz and linear probes (Gufler *et al.*, 1998; Franz *et al*.,2001; Gungor *et al*., 2005; Fasulkov, 2012). One of the biggest advantages of water bath method is that it allows the operator to use one hand to move the teat in different positions and manipulate the sonograph, while at the same time holding the plastic cup and moving the probe vertically. Using of this method for displaying the internal structures of the teat prevents deformations in its tip, which would impede the visualization of the papillary canal along its entire length (Franz *et al*., 2009). Fasulkov (2012) also reported, that the application of the water bath technique was more effective for examining the teat, whereas the transcutaneous method (direct contact) – in the examination of the structures in the other areas of the mammary gland (Fasulkov *et al*., 2010; Fasulkov *et al*., 2010; Fasulkov & Koleva, 2011). The last usable method for ultrasonographic examination of the teat is called "stand of technique". Twardon *et al.* (2001) and Santos *et al.* (2004) performed ultrasonography of teats by dipping in liquid and using liquid pressure, respectively. Hoque *et al.* (2004) mentioned that the scanning of superficial structures poses a problem by obscuring the image due to near field reverberations and suggested the use of a commercially available stand-off pad or home-made fluid filled plastic bag /condom to evaluate superficial structures (less than 3.5 cm depth). Rambabu *et al.* (2008) used a gel filled condom as a standoff, which provided satisfactory results.

The interpretation of the ultrasound image

The ultrasound image of the teat in cows has been described by many authors (Jenninger,1989; Klein *et al*., 2001; Neijenhuis *et al*., 2001; Weis *et al*., 2004; Flock and Winter, 2006; Stádník *et al*., 2010). Ultrasonography with the water bath method and vertical scanning of the teat with higher frequency probes are most commonly used. Generally, the higher frequency probes can capture more detail images of the scanned surface, but their penetration depth is limited. The findings of Franz *et al*. (2001) on the teat canal ultrasonography in cows indicated that a linear probe with 8.5 MHz, made higher quality images, whereas Couture & Mulon (2005) believed that probes with frequencies of 5 MHz, 7.5 MHz and 10 MHz could also be used for this purpose.

The normal appearance of the udder and teat are amenable to sonographic imaging because of their superficial location and the technique has the potential to diagnose different conditions of the organ. Rambabu *et al.* (2008) reported that the glandular parenchyma of udder on ultrasonographic examination appeared as homogenous and hyperechoic with anechoic alveoli, which was in accordance with Ayadi *et al.* (2003) in cows and Gungor *et al*. (2005) in mares. The gland sinus appears as homogenous anechoic area whereas Cartee *et al.* (1986) observed the gland sinus as an anechoic area continuous with the teat sinus. The lining of wall of the gland sinus appears as mixed hyper-hypothetic folds. The lactiferous ducts are anechoic areas within

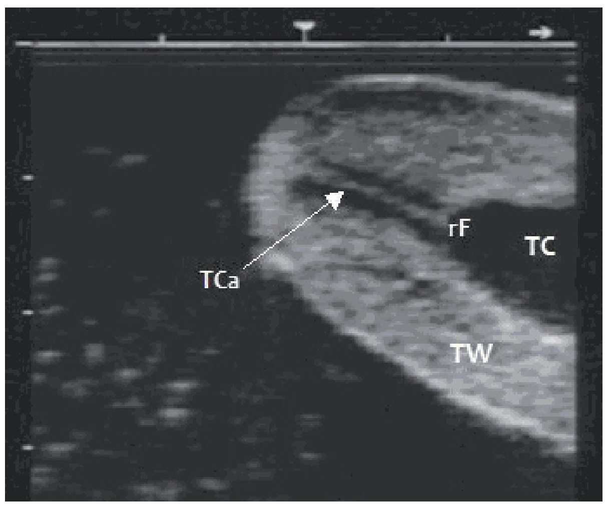

Fig. 1: Ultrasonographic appearance of cow teat with the water bath method. (vertical scan, 7.5 MHz linear probe) : TCa – teat canal; TW – teat wall; TC – teat cistern; rF – rosette of Fustenberg.
Photo: own data

the hypothetic matrix of the fold. Gungor *et al.* (2005) described the lactiferous duct as an elongating anechoic branches in hyper-echoic mammary parenchyma. Some of the anechoic areas within the glandular parenchyma may have been blood vessels but others certainly were lactiferous ducts, because they could be seen entering the gland sinus.

In the sonographic image, the teat wall appears as a threefold layered structure. The teat skin appears as a 1–2 mm thin, bright, echoic line and is followed by the muscular/connective tissue layer containing blood vessels showing a thicker, homogeneous, less echoic layer with inclusion of anechoic cavities. The internal boundary as mucous membrane appears as a thin bright line (Hospes & Seeh, 1999; Franz *et al.* 2001). Physiological teat canal sonograms are presented as a thin, white, hyperechoic line circumscribed on each side by parallel hypo- to anechoic bands. Franz *et al.* (2001) compared these sonographical findings with histological preparations. They found that stratum corneum with keratin causes the hyperechoic middle line, and the stratum granulosum the surrounding less echoic lines.

Using a 7.5 MHz linear probe, Franz *et al*. (2001) described the teat canal as a thin, hyperechoic line between two thicker paralel hypoechoic bands, which was histologically distinguished as the hypoechoic papillary layer, and the hyperechoic line of the stratified keratinized pavement epithelium which included the keratin stratum. The transition between the teat canal and the teat cistern is designated as the rosette of Furstenberg (Riedl *et al.*, 2004). Furstenbergs rosette appears as 2-5 parallel, short hyperechoic lines extending from the teat canal into the teat sinus (Khol *et al*., 2006). The teat cistern is anechoic, surrounded by a hyperechoic line (mucosa) (Takeda, 1989; Sendağ & Dinc, 1999; Santos *et al.*, 2004), it could not be visualised if not filled with milk (Franz *et al*., 2001). The boundary between the gland and teat cisterns is visualized as round anechoic structures, which correspond to the veins of the venous ring of Furstenberg (Franz *et al.*, 2009).

Practical use of ultrasonography and its clinical application

As we mentioned before, udder and teat scanning is generally performed for diagnosis of milk flow disturbances, which are mostly caused by teat injuries and milking technique (Klein *et al*., 2005; Geishauser & Querengässer, 2000) Ultrasonographic scanning

of the teat is used primarily on cows and sheep for the diagnostics of obstructions, stenoses, and the rosette

of Furstenberg, fibrous changes in the area of the teat canal, or the boundary between the teat and gland cisterns (Trostle & O'Brien, 1998; Dinc *et al.*, 2000; Couture & Mulon, 2005; Fasulkov, 2012). Ultrasonography is used for examination of the mammary gland parenchyma in cases of inflammatory processes (mastitis), pathological formations localized deep within the parenchyma (abscesses, haematomas, tumours, foreign bodies, connective tissue buildups) oedema of the udder without mastitis symptoms, which cannot be detected by clinical examination (Flock & Winter, 2006; Franz *et al*., 2009).

Another use of ultrasonography of the udder is for designation of the internal teat structures. It allows detailed measurements of the length and diameter of the teat canal, cistern, and the thickness of the teat wall (Gleeson *et al*., 2002; Ślosarz *et al*., 2010, Stándík *et al*., 2010). Many authors found a relation between mastitis in cows, the characteristics of the teat, the stage of lactation and the visualization of the teat canal (McDonald, 1975; Seykora & McDaniel, 1985; Grindal *et al*., 1991; Seyfried, 1992; Celik *et al*., 2008).

Many different external and internal parameters such as breed (Klein *et al*., 2005), number of lactations (Hamann, 1988), duration of lactation (Mcdonald, 1973), teat shape (Hebel, 1978) and milking technology (Hamann *et al*., 1994; Neujenhuis *et al*., 2001) influence the morphology of the tissue of the bovine teat and teat canal. During machine milking, tractive and shear forces caused by vacuum and weight of the teat tip and teat canal (Hamann *et al*., 1994), lengthening and increasing concerning the thickness of the teat canal (Neijenhuis, 2004), as well as the loss of keratin followed by reformation of the keratin layer (Hamann & Burvenich, 1994; Khol *et al*., 2006). Differing results regarding thickness of the teat canal and its length are caused by various procedures used as well as by lactation, age of animals and by different time of examination (Khol *et. al.*, 2006). Basing on these facts, the ultrasonography can be useful in reviewing breed differences in the number of lactations, the teat canal elongation as a consequence of mechanical milking, and determining the influence of the teat parameters on the udder health status (Fasulkov, 2012).

CONCLUSION

In conclusion, ultrasonography is an easy applicable, modern and noninvasive method, allowing the visualization of the mammary gland (teat and parenchyma) and their morphological changes. Also the largest advantages of sonography are a relatively simple and effective use, safe to the subject and the operator, its portability and fast interpretation and diagnosis in most circumstances. The main indications for the use of this method include milk secretion disorders, diagnostics of pathological alterations of the udder (inflammation, mucosal lesions, foreign bodies, congenital changes, hematoma and abscess etc.), measurements of teat canal length and diameter, teat cistern diameter, and teat wall thickness.

Currently, numerous studies have been published using ultrasonography as an effective measuring technique for teats. Most of them focused on recovery of teats and teat–end penetrability after milking which is important for udder health. However, case-control research is needed to prove differences in reaction of teats to milking among and within cows due to milk flow. Evaluation of teat parameters by ultrasound measurement can be a good help in this research.

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