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## ULTRASONOGRAPHY OF THE MAMMARY GLAND IN RUMINANTS: A REVIEW

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## Summary

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Fast and accurate diagnostics and prognosis are very important with regard to mammary gland illnesses in ruminants due to the negative economic impact of the loss of milk productivity. This necessitates the usage of modern, accurate and quick methods for mammary gland examination, such as ultrasonography. The main advantage of ultrasonography examination is the method's noninvasiveness and the possibility for visualisation of the structures in all parts of the mammary gland (teat and parenchyma). Possible indications for application could be disruptions in milk secretion, diagnostics of pathological changes (stenosis and obstructions, inflammations, abscesses, haematomas, foreign bodies, etc.), measurements of teat canal length and diameter, teat cistern diameter, and teat wall thickness. The ultrasound examination of the mammary gland parenchyma in ruminants is mainly performed through the direct contact technique (transcutaneous echography) with lowfrequency linear, sector or convex transducer (3.5-5 MHz) and horizontal scanning. Examination of the teat is most commonly conducted through the water bath technique and vertical scanning. A highquality image can be produced if a high-frequency probe is used. The present review aimed to summarise the data related to ultrasound examination of the mammary gland in ruminant animals, with regard to its advantages, clinical applications, indications for performance, scanning techniques, and the interpretation of the echographic image of the udder's different parts.

Key words: ultrasonography, mammary gland, ruminants

#### INTRODUCTION

Maintaining a healthy mammary gland in ruminants plays a significant role in modern animal husbandry aimed at achieving high-quality milk production (Wendt *et al.*, 1994; Kossaibati, 2000). 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 in the quality of the 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, the quick and accurate diagnostics and prognosis are exceptionally important in mammary gland disorders. This entails the application of modern, accurate and fast methods for examination, such as ultrasonography.

Ultrasonography is a non-invasive method for the diagnostics of various physiological and pathological conditions of the reproductive organs of ruminants (Dimitrov *et al.*, 2002; Kähn, 2004; Yotov, 2005; Vassilev *et al.*, 2005; Yotov *et al.*, 2008). Ultrasound examination of the mammary gland in ruminants was performed for the first time by Caruolo & Mochrie (1967), who studied the teats of lactating cows with an A-mode ultrasonic device and a 1 MHz probe. The first report of applying B-mode ultrasonography to a mammary gland was done by Cartee *et al.* (1986). They used a 5 MHz linear and 5 and 10 MHz sector transducers to determine milk secretion distrubances and structural changes in the teat of cows.

During the 1970s and the 1980s, teat radiography was routinely used in the diagnostics of milk secretion anomalies (Kubicek, 1972; Witzig & Hugelshofer, 1984; Witzig et al., 1984; Alacam et al., 1990). In more recent times, however, this method of visualisation of mammary gland structures has been replaced by ultrasonography. In support of this statement, Stocker & Rüsch (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. Teat endoscopy (theloscopy) permits a direct visualisation of pathological changes (Couture & Mulon, 2005). Unlike ultrasonography, the loscopy only allows 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; Vangroenweghe et al., 2006; Kiossis et al., 2009).

A number of authors have reported about mammary gland ultrasonography in cows (Jenninger, 1989; Stocker *et al.*, 1989; Will *et al.*, 1990; Bruckmaier & Blum, 1992; Saratsis & Grunert 1993; Seeh *et al.*, 1996; Banting, 1998), sheep (Ruberte *et al.*, 1994; Nudda *et al.*, 2000; Franz *et al.*, 2003; Wójtowski *et al.*, 2006; Rovai *et al.*, 2008; Hiepler *et al.*, 2009) and goats (Wójtowski *et al.*, 2002; Fasulkov *et al.*, 2010; Ślósarz *et al.*, 2010). Ruberte *et al.* (1994) and Franz *et al.* (2003) used the method to visualise the parenchymal tissue, the mammary canals, the gland and teat cisterns, and the teat wall in sheep. Using 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.

Echography of the teat is used for detection and differentiation of stenosis or obstruction of the teat canal, tissue proliferation, foreign bodies, milk stones, and congenital defects (Hospes & Seeh, 1999; Dinç *et al.*, 2000; Franz *et al.*, 2009). Echography can be used to monitor the recovery process after surgical removal of proliferated tissue or to differentiate a supernumerary teat or fistula (Franz *et al.*, 2009). It is also used in the diagnostics of mastitis, haematomas, abscesses and tumours of the mammary gland (Gonzalez de Bulnes *et al.*, 1998; Flöck & Winter, 2006; Trasch *et al.*, 2007).

Apart from the studies on the application of B-mode echography, reports on the latest methods of ultrasound examination of the mammary gland in ruminants, such as colour Doppler echography (Franz *et al.*, 2001; Braun & Hoegger, 2008) and three-dimensional (3D) echography (Franz *et al.*, 2004) are also available.

## TECHNIQUES OF EXAMINATION AND ULTRASOUND IMAGE INTERPRETATION

#### Techniques of examination

Using a proper B-mode ultrasonography equipment allows the differentiation of

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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 transducers can be used (Cartee *et al.*, 1986; Flöck & Winter, 2006; Wójtowski *et al.*, 2006).

Ultrasound examination of the mammary gland parenchyma in ruminants and the space between the gland and teat cisterns is done primarily through the direct contact technique (transcutaneous echography, Fig. 1) with a 5 MHz or lower frequency transducer (Cartee et al., 1986). After preliminary hair shaving, dipping of the skin with alcohol and contact gel application, the transducer is placed on the lateral and caudal surfaces, directly on the udder skin (Franz et al., 2009). During scanning, the probe is placed longitudinally and transversely to the mammary gland, designated as horizontal and vertical position, respectively (Cartee et al., 1986; Franz et al., 2003; Güngör et al., 2005).



Fig. 1. Ultrasonography of a goat's teat through the direct contact technique.





Fig. 2. Ultrasonography of a goat's teat through the water bath technique:A – horizontal scan; B – vertical scan.

The primary technique used for echography of the teat structures is the "water bath" (Stocker et al., 1989; Will et al., 1990; Saratsis & Grunert, 1993; Şendağ & Dinç, 1999; Santos et al., 2004; Franz et al., 2009). It is done by immersion of the teat in a plastic container filled with warm water (30–35°C), with the transducer placed horizontally (Fig. 2A) or vertically (Fig. 2B) in contact with the container (Stocker et al., 1989). 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 visualisation using the water-bath technique. The

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echographic examinations of the mammary glands of lactating goats performed by us showed that transcutaneous teat echography (direct contact) using a linear 5 MHz transducer did not produce satisfactory results (Fasulkov *et al.*, 2010). For high quality of the echographic teat image, most authors recommend using a linear probe with a frequency of no less than 7.5 MHz (Gufler *et al.*, 1998; Franz *et al.*, 2001; Güngör *et al.*, 2005; Flöck & Winter, 2006).

Franz *et al.* (2009) believed that the water bath technique allows the operator to use one hand to move the teat in different positions and manipulate the echograph, while at the same time holding the plastic container and moving the probe vertically. The authors consider that using this method for displaying the structures of the teat prevents deformations in its tip, which would impede the visualisation of the papillary canal along its entire length.

Şendağ & Dinç (1999) observed a better image when the teat was full of milk or if neutral solutions were introduced before the examination. In support of this claim, Franz *et al.* (2001) proved that it was not necessary to fill the teat with sterile water or saline solution while examining animals in their lactation period.

Our studies with lactating goats showed 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.*, 2011; Fasulkov & Koleva, 2011).

Another method for echographic examination of the teat is the "stand off" technique (Fig. 3). Rambadu *et al.* (2008) used this technique in buffaloes by placing the teat in a latex condom filled with con-



**Fig. 3.** Ultrasonography of a goat's teat through the stand off technique.

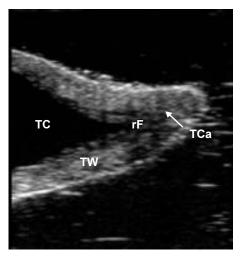
tact gel, with the transducer moved vertically or horizontally along the outer surface of the condom.

## Ultrasound image of the teat

The ultrasound image of the teat in ruminants has been described by a number of authors (Cartee *et al.*, 1986; Stocker *et al.*, 1989; Takeda, 1989; Trostle & O'Brien, 1998; Nudda *et al.*, 2000; Franz *et al.*, 2001; Babkine & Couture, 2002).

B-mode echography of the teat in ruminants is most often performed using the water bath technique and vertical scanning (Stocker *et al.*, 1989). The data by Franz *et al.* (2001) on teat canal echography in cows and sheep indicate that a highquality image of the teat can be produced by using a 8.5 MHz linear transducer and the vertical position, 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.

Our results on the echography of goat teats using different techniques indicated that the water bath technique provided the best option for observation of the goat's teat structures (Fig. 4), while using a 10 MHz probe provided a high-quality image (Fasulkov *et al.*, 2011). The direct contact and stand off techniques allowed the visualisation of the proximal papillary part, yet observation of the structures within the distal part of the teat was considerably limited (Fasulkov *et al.*, 2011).



**Fig. 4**. Visualisation of the structures of the goat's teat (water bath technique, vertical scan, 10 MHz linear probe): TCa – teat canal; rF – rosette of Furstenberg; TW – teat wall; TC – teat cistern.

Cartee *et al.* (1986) observed the papillary orifice as a small anechoic formation located at the tip of the teat, whereas Şendağ & Dinç (1999) and Couture & Mulon (2005) established it as a hyperechoic structure.

Using a 3.5 MHz linear transducer, Stocker *et al.* (1989) visualised the teat canal as a single hypoechoic zone. Unlike them, Will *et al.* (1990) and Saratsis & Grunert (1993), using a 5 MHz linear probe, visualised the teat canal as one or two hyperechoic areas, whereas Franz *et al.* (2001) observed it via an 8.5 MHz linear probe as a centrally located hyperechoic line, bordered by two parallel hypoechoic zones.

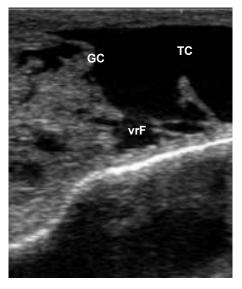
The transition between the teat canal and the teat cistern is designated as the rosette of Furstenberg (Riedl *et al.*, 2004). During echographic examination, it is visualised as a homogenous hyperechoic structure located directly above the teat canal (Franz *et al.*, 2009).

By ultrasonography, Cartee et al. (1986), Şendağ & Dinç (1999) and Franz et al. (2003), differentiated three distinct layers of the teat wall in cows and sheep. The outer layer (teat skin) appears as a lucent hyperechoic line. The middle layer (musculature and connective tissue) is homogenous, thick, and of moderate echogenicity (hypoechoic layer), while the innermost layer (the mucosa) is hyperechoic. Our echographic studies on lactating goats also proved three different layers of the teat wall (Fasulkov et al., 2010). Couture & Mulon (2005) differentiated 5 papillary wall layers depending on the echogenicity - skin, hyperechoic musculature, connective tissue and submucosa with average echogenicity, and hyperechoic mucosa.

The teat cistern is anechoic, surrounded by a hyperechoic line (mucosa) (Takeda, 1989; Şendağ & Dinç, 1999; Santos *et al.*, 2004), yet it could not be visualised if not filled with milk (Franz *et al.*, 2001). The boundary between the gland and teat cisterns (Fig. 5) is visualised as round anechoic structures, which correspond to the veins of the venous ring of Furstenberg (Franz *et al.*, 2009).

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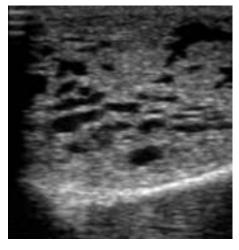


**Fig. 5.** Sonogramme of the transition between the teat and gland cisterns of a goat (direct contact technique, vertical scan, 5 MHz linear probe): TC – teat cistern; GC – gland cistern; vrF – venous ring of Furstenberg.

# Ultrasound image of the mammary parenchyma

The echographic examination of the mammary gland parenchyma in ruminant animals is performed primarily via the direct contact technique (transcutaneous echography). For this purpose, a linear, convex or sector transducer can be used, with a frequency of 3.5 and 5 MHz (Cartee *et al.*, 1986; Trostle & O'Brien, 1998; Flöck & Winter, 2006).

The echogenicity of the lactating mammary gland depends on its volume (Cartee *et al.*, 1986; Flöck & Winter, 2006). According to Franz *et al.* (2009), the cow's mammary gland parenchyma is a homogenous structure and the visualisation of anechoic zones within it is usually related to a blood vessel or a lactiferous duct. The milk in the gland cistern is an anechoic formation (Fig. 6) with the presence of echoic particles.



**Fig. 6.** Sonogramme of goat's mammary gland parenchyma (direct contact technique, horizontal scan, 5 MHz linear probe).

Cartee *et al.* (1986) established the gland cistern in cows as a large anechoic zone, whereas the parenchyma was hypoechoic. Our results during the echographic examination of lactating goats were similar. We proved that the lack of milk secretion makes the visualisation of the udder structures more difficult. This necessitates the separation of the offspring in goats kept together with the newborn, at least two hours before the examination. For other animals, a better visualisation can be achieved when the examination is performed at least 2 hours after the last milking (Fasulkov *et al.*, 2010).

Flöck & Winter (2006) established the parenchyma of a healthy mammary gland as a homogenous structure of average echogenicity and presence of anechoic blood vessels and lactiferous ducts. The authors observed an abscess within the mammary parenchyma as a round clearly distinguished structure with a different size, a visible capsule and hypoechoic content. Haematomas were displayed as large septated areas within the gland parenchyma, filled with anechoic to hypoechoic fluid (Flöck & Winter, 2006; Franz et al., 2009).

# INDICATIONS AND CLINICAL APPLICATION OF THE METHOD

Milk secretion disturbances are the main indication for ultrasound examination of the mammary gland in ruminants (Franz *et al.*, 2009). Teat injuries and the milking technique are the main reasons for disturbed milk secretion (Bleul *et al.*, 2002; Geishauser *et al.*, 2005; Condino *et al.*, 2010).

Echographic scanning of the teat is used primarily on cows and sheep for the diagnostics of stenoses, obstructions and fibrous changes in the area of the teat canal, the rosette of Furstenberg or the boundary between the teat and gland cisterns (Saratsis, 1991; Trostle & O'Brien, 1998; Dinç *et al.*, 2000; Flöck *et al.*, 2004; Mavrogianni *et al.*, 2004; Couture & Mulon, 2005).

Echography is used for examination of the mammary gland parenchyma in cases of inflammatory processes (mastitis), oedema of the udder without mastitis symptoms, pathological formations localised deep within the parenchyma (abscesses, haematomas, tumours, foreign bodies, connective tissue buildups), which cannot be detected through clinical examination (Flöck & Winter, 2006; Franz et al., 2009). Banting (1998) used the echostructure of the mammary gland in cows with induced staphylococcal mastitis (Staphylococcus aureus), in order to determine the prognosis and the choice of treatment. Flöck & Winter (2006) found out the characteristic echographic image in cows' mammary glands inflammation caused by Arcanobacterium pyogenes and some Gram-negative bacteria of the Enterobacteriaceae family. They described the changes in the udder parenchyma in cases

of abscesses and haematomas. Franz *et al.* (2009) observed a penetrating foreign body in the parenchyma as a hyperechoic linear structure causing a strong acoustic window. O'Brien *et al.* (2002) found gases during the echographic examination of an oedematous mammary gland in cows.

Our echographic studies on the mammary gland in goats suffering from acute mastitis, revealed a non-homogenous and hypo- to hyperechoic mammary gland parenchyma structure, with a lack of clear visualisation of the mammary canals and the blood vessels (Fasulkov & Koleva, 2011). The echography of the teat in these animals exhibited a thickened hyperechoic teat wall, as well as numerous hyperechoic structures in the teat cistern, representing milk coagula. Because of the more severe clinical expression and the major change in the echogenicity of the udder of goats with acute mastitis caused by Staphylococcus aureus, we believe that the severity of the inflammation and the type of the etiological agent possibly affected the echographic image for these animals (Fasulkov & Koleva, 2011).

Another indication for the echographic examination of the mammary gland is the determination of the dimensions of structures within the area of the teat. It allows for detailed measurements of the length and diameter of the teat canal, cistern, and the thickness of the teat wall (Gleeson et al., 2002; Ślósarz et al., 2010). Spencer et al. (1996), Gleeson & O'Callaghan (1998) and Neijenhuis (1999) used echography to detect changes in the teat tissues of cows. A number of authors established a connection between mastitis in cows, the stage of lactation, the characteristics of the teat, and the visualisation of the teat canal (McDonald, 1975; Seykora & McDaniel, 1985; Grindal et al., 1991; Seyfried, 1992; Scherzer, 1992; Celik et al., 2008). Some studies have pointed out that echography 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 development of mastitis (Neijenhuis *et al.*, 2001; Ayadi *et al.*, 2003; Klein *et al.*, 2005; Khol *et al.*, 2006; Wójtowski *et al.*, 2006; Rovai *et al.*, 2008; Olechnowicz & Jaśkowski, 2009).

## CONCLUSIONS

In conclusion, it could be summarised that ultrasound imaging of the mammary gland in ruminants is a non-invasive method, allowing the visualisation of the separate structures of the mammary gland (teat and parenchyma).

The main indications for application of this method inlcude milk secretion disorders, diagnostics of pathological changes (stenosis and obstructions, inflammations, abscesses, haematomas, foreign bodies, etc.), measurements of teat canal length and diameter, teat cistern diameter, and teat wall thickness.

Echography of the mammary gland parenchyma in ruminants is performed primarily through the direct contact technique (transcutaneous echography) with a low-frequency linear, sector or convex transducer (3.5–5 MHz) and horizontal scanning.

Examination of the teat is done primarily through the water bath technique and vertical scanning. A high quality image can be achieved by using a high frequency probe (at least 7.5 MHz).

The application of ultrasonography for the diagnostics of physiological and pathological conditions of the mammary gland has been commonly reported for cows and sheep, whereas data about goats are scarce. This is a rationale for future echographic studies on various pathological conditions of the mammary gland in goats, aimed at correct diagnostics and monitoring of goat mammary gland pathology.

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