ULTRASONOGRAPHY AS A DIAGNOSTIC TOOL IN RUMINANT VETERINARY MEDICINE: APPLICATIONS IN THE MAMMARY GLAND

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ABSTRACT

Brazilian livestock stands out as one of the most important in the world, with Brazil being the third largest producer of milk in the world, currently producing more than 34 billion liters/year. Thus, rapid diagnosis and prognosis is necessary for the early and/or subclinical identification of pathologies that may interfere with dairy farming, directly impacting the Brazilian economy. This requires mastery of the clinical examination of the mammary gland, which must be performed through thorough and complete inspection and palpation, in addition to the use of modern, precise and fast methods, such as ultrasound. Ultrasound of the mammary gland has been highlighted for being a non-invasive method that allows the visualization and measurement of different structures (length and width of the teat canal, diameter of the cistern and thickness of the teat wall) and pathological changes (gas formation, obstructions, hematomas, abscesses, stenosis and fibrosis). The most commonly used technique is direct contact scanning with low-frequency linear, convex, and microconvex transducers of 2.5–6.6 MHz and water bath scanning. However, the technique of immersion in a water bath has been used when it is desired to meticulously evaluate the structures of the ceiling, as it produces a high-quality image. Thus, the use of ultrasound presents itself as a practical and efficient tool, enabling the identification of alterations in the mammary gland and contributing to both the prevention and treatment of diseases, being of great relevance in the area of Veterinary Medicine.

Keywords: Dairy cattle. Pathologies. Udder. Ultrasound.

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1 INTRODUCTION

Brazilian agribusiness plays a crucial role in the country's economy, standing out in the dairy sector. As a world milk producer, Brazil is the third largest, with an average growth rate of 4% per year, currently producing more than 34 billion liters. Due to this increase in production and intensification of dairy technology, it is essential to know the morphophysiology and health of the udder (Ministry of Agriculture and Livestock, 2023).

There is a strong genetic correlation between milk production and udder type characteristics. It is possible to increase production and reduce udder-related disorders such as mastitis by selecting cows based on the optimal udder type attributes. Milk production is an important selection criterion in dairy cattle breeding. The cow's udder is one of the most important criteria used to predict performance. Type traits are recorded relatively early in life and are hereditary (Brito *et al*., 2021).

2 ANATOMY OF THE MAMMARY GLAND:

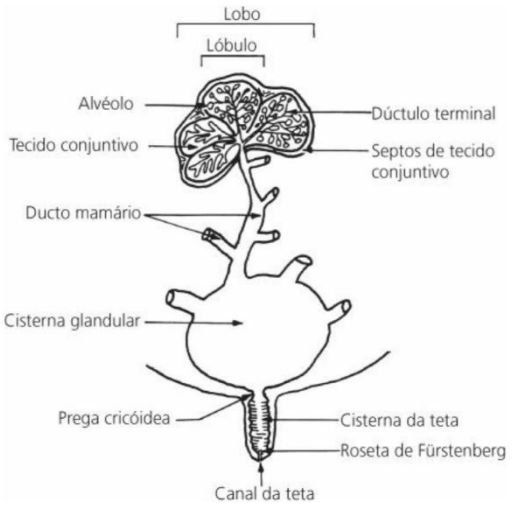
Anatomically, the udder of the bovine female is formed by four individual and independent mammary glands that are consolidated in a single mass, the udder, fixed in the inguinal region and extending between the thighs. The four glands are divided into mammary quarters, two anterior, right and left, and two posterior, right and left. The posterior mammary quarters are the ones that produce the most milk and are significantly larger in size compared to the anterior ones (Bragulla; König, 2014; Feitosa, 2020).

Its support system allows cows to carry large amounts of milk, and it is composed of the suspensory ligaments of the mammary gland, of which the lateral suspensory ligament of the breast, the middle ligament, the connective tendons and the connective tissue that lines the gland stand out (Bragulla; König, 2014; Ribeiro *et al.,* 2021).

Histologically, the mammary gland is classified as a modified sweat gland that synthesizes milk in a single layer of secretory epithelial cells. These morphofunctional units that produce, store and secrete milk. This milk, after being produced by the secretory cells, is drained from the main ducts to the cistern of the gland, where it is retained (Machado, 2021).

The cistern of the gland communicates with the cistern of the roof through a circular ridge (annulus) that contains a vein and some fibers of smooth muscle. The roof cistern connects to the outside via a papillary duct (ceiling canal) that opens into the papillary ostium. Milk retention is mainly controlled by a muscle sphincter that surrounds the teat canal (Machado, 2021; Ribeiro *et al.,* 2021).

Figure 1. Duct and lobuloalveolar systems of the bovine mammary gland.



Source: Reece, (2017)

Currently, the morphological evaluation of the mammary gland of dairy cows has been widely performed. This fact is due to the increase in demand and intensification of dairy technologies, with the objective of finding an anatomical pattern for the selection of animals with higher production and udder health, because by identifying these ideal morphological traits, these animals can be selected, on the other hand some traits are used as discard factors (Castañeda-Bustos *et al*., 2017; Teissier *et al*., 2019).

3 PHYSIOLOGY OF THE MAMMARY GLAND

Milk-secreting cells make up the parenchyma of the mammary gland, developing through the proliferation of epithelial cells that arise from primary mammary cords. Epithelial cells form hollow, circular structures called alveoli, which are the milk-secreting units (Klein, 2014; Machado, 2021).

Some hormones, such as estrogen (E2), progesterone (P4), somatotropin (GH) and prolactin (PRL), influence the growth of the glandular parenchyma, and prolactin and progesterone also act directly in the multiplication of the mammary alveoli. Cortisol at the end of pregnancy undergoes a large increase, where it will influence the growth of the mammary gland. Estrogen (E2) and prostaglandin (PGF2 α) also have an influence on the gland (Braga *et al*., 2015; Feitosa, 2020; Machado, 2021). High progesterone levels in late pregnancy prevent lactogenesis. Reducing the level of this hormone shortly before calving allows the lactogenic complex to initiate cell differentiation and lactation (Reece, 2017; Feitosa, 2020).

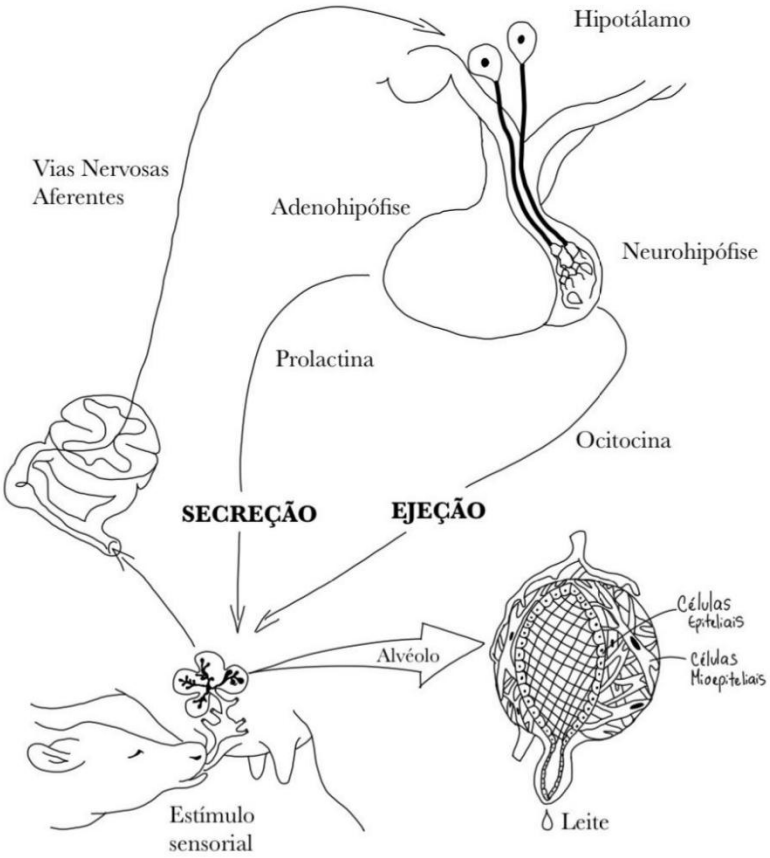
At the beginning of lactation, alveolar cells undergo remarkable maturation of the rough endoplasmic reticulum, smooth endoplasmic reticulum, and Golgi apparatus under

the influence of PRL and glucocorticoids, resulting in the cellular ability to synthesize and secrete protein, fat, and lactose (Reece, 2017). Upon stimulation in the teat, basal secretion of dopamine occurs by the hypothalamus, as it acts as a prolactin inhibitor factor in high concentrations, however, its low serum concentration causes the hypothalamus to secrete Vasoactive Intestinal Peptide (IPV) to stimulate the pituitary gland. Then, the adenohypophysis will release PRL, which in turn will act directly on the alveolar cells, so that they synthesize milk (Braga *et al*., 2015; Reece, 2017).

The synthesis of milk requires a large amount of nutrients, which originate in the bloodstream, where it is estimated that it is necessary to pump 500L of blood to the mammary gland for each liter of milk (Santos *et al*., 2007). Understanding the process of synthesis and ejection of milk through sensory stimuli of the calf or milking is extremely important in productivity. As for milk production, the calf when suckling or milking when sucking on the mammary gland will generate sensory stimuli that will be carried through ascending nerve pathways from the spinal cord to the hypothalamus, causing dopamine inhibition, PRF stimulation and prolactin production through the adenohypophysis. Prolactin, in turn, will produce milk in the udder (Braga *et al*., 2015; Klein, 2017; Ribeiro *et al*., 2021).

Regarding milk ejection, the sensory stimulus will travel the same path, but when it reaches the hypothalamus, oxytocin will be produced by the neurohypophysis, causing oxytocin to reach the myoepithelial cells of the mammary gland, performing the contraction of the alveoli and consequently the ejection of milk (Reece, 2017; Klein, 2017; Ribeiro *et al*., 2021).

Figure 2. Physiology of milk synthesis and secretion by sensory stimuli of the calf or in milking



Source: Adapted from Ribeiro (2021)

4 CLINICAL EXAMINATION OF THE MAMMARY GLAND:

According to the literature, the 4 (four) initial items of the examination plan can be cited as: identification of the animal (name, record, species, breed, characteristics, sex, weight, age and owner), anamnesis or history, examination of vital functions (respiratory rate, rumen rate, heart rate, temperature, appetite, defecation, urination, mucous membrane coloration) and evaluation of the patient's general condition (temperament, state of nutrition, attitude in season, locomotion and in decubitus) (Birgel, 2014; Feitosa, 2020; De Bonis, 2023).

The accurate diagnosis of diseases that affect the mammary gland, especially the clinical forms of mastitis in domestic milk-producing animals, are responsible for considerable economic losses to dairy farming and requires a thorough and complete clinical examination (Birgel, 2014; Feitosa, 2020). However, it is necessary to establish two preliminary conditions that must be routinely followed and obeyed in the day-to-day practice of the veterinary clinic: the clinical examination plan of the mammary gland and the mastery of mammary gland semiology (Feitosa, 2020; Porn *et al*., 2024 a).

The initial stage of the clinical examination of the mammary gland consists of inspection, which aims to identify changes in development, support, and number of teats. Asymmetries in size and shape, increase in volume, atrophy or hypertrophy of one or more mammary quarters, as well as the presence of protuberances, such as abscesses, usually associated with mastitis, should be observed. In addition, it is essential to evaluate udder insertion, changes in coloration, such as hyperemia, and possible skin lesions, such as abrasions, crusts, vesicles, ulcers, and pustules (YAGÜE *et al.*, 2014; Feitosa, 2020; De Bonis, 2023).

Palpation of the entire breast parenchyma should be performed in each mammary quarter, following the caudal-cranial and dorsal-ventral directions, as described by Feitosa (2008), which is performed to investigate the temperature, sensitivity and consistency of the different structures of the mammary gland. Before starting palpation, the elasticity of the skin and subcutaneous tissue is checked by means of a pleat, under normal conditions it is easy to pleat the skin, and once the pressure ceases, it quickly returns to natural conditions (Feitosa, 2020; De Bonis, 2023). According to Feitosa (2020), in edemas, both physiological, which affect the mammary gland before and/or immediately after childbirth, and in inflammatory edema, there is no possibility of pleating the skin, and once the pressure is eliminated, a depression is clearly perceived in this place, with what is called *a positive Godet* test. This method of clinical exploration also evaluates the sensitivity of the mammary gland, which will be increased, in a variable degree of intensity, in acute mastitis (YAGÜE *et al.*, 2014; Feitosa, 2020; Porn *et al*., 2024b).

On the other hand, the inspection of the teats shows size, shape, supramammary teats, and position. The nozzles should be at least 40 to 45 cm from the ground, their shape should be rounded or hemispherical. The opening of the teat canal should be in the center of the beak, and not to one side. Palpation should be performed with the fingertips, and reveals any tumor, with or without an increase in temperature, as well as lesions, fistulas, granules, nodules, diffuse hardening, acute edema, elastic consistency. These changes cause a drop in milk production and for this reason, rapid and accurate diagnosis and prognosis are exceptionally important in mammary gland disorders (Yague *et al.*, 2014; Feitosa, 2020; Rosenberger *et al*., 2020).

5 ULTRASOUND APPLIED TO THE MAMMARY GLAND

Lately, ultrasonography has proven to be an increasingly attractive tool for assessing tissue development and specific body structures. Among its main advantages, it is worth highlighting the fact that it is a non-invasive technique that allows longitudinal evaluations in the same animal throughout the development process, in addition to real-time visualization

and assistance in the identification of pathological changes (Nishimura *et al*., 2011; Seibt *et al*., 2023).

The ultrasound technique is based on the emission of sound waves with a frequency of 2-7.5 MHz, and when they encounter a barrier, they are reflected by the tissues in the form of echoes, which are captured back, forming an image (Braga *et al.,* 2015; Themistokleous *et al*., 2023). However, it allows for a more accurate assessment of the udder morphology of lactating animals and offers the possibility of obtaining a detailed view of the different structures and characteristics of the tissues, in order to differentiate and define them, without the need for dissection (Fasulkov, 2012; Braga *et al.,* 2015; Seibt *et al*., 2023).

According to the authors, through ultrasound examination, it is observed if there is gas formation, hematomas, abscess, tumors and changes in the echogenicity of the breast parenchyma when there is an infection installed. The accurate diagnosis of the presence of abscess in mastitis cases, when these are not perceptible on palpation and do not communicate with the external environment through fistulas, is of great relevance, because it can explain the recurrence or maintenance of the infectious condition in a given animal (Fasulkov, 2012; De Bonis, 2023). In addition, among other features, it also makes it possible to carry out a detailed and accurate measurement of the length and width of the channel and diameter of the cistern and thickness of the ceiling wall. However, it is essential to establish a standard of physiological measurements and to understand the changes caused by lactation in these parameters, so that this standard can be used as a reference for a reliable diagnosis and adequate prognosis and early treatment (Franz *et al*., 2009; Fasulkov, 2012; Braga *et al*., 2015; Ribeiro *et al*., 2021).

To obtain images of the mammary gland structures, previous knowledge of ultrasound scanning techniques is required, the main ones being direct contact and water bath techniques. In general, the images are obtained by means of a B-mode ultrasound device, equipped with a linear or microconvex probe, as in the experiments by Seibt *et al*. (2023) and Albino *et al*. (2017).

6 SCANNING BY DIRECT CONTACT TECHNIQUE

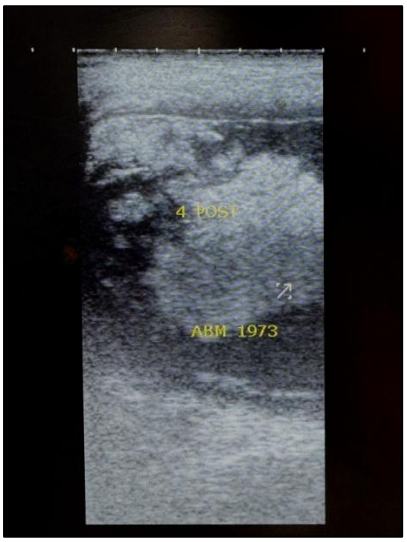
This technique is the most widely used technique for evaluating the parenchyma gland and the space between the gland and the roof cistern systems, in which a linear, sectoral, or convex transducer with a frequency of 2.5–6.6 MHz is used, by applying alcohol or contact gel to the region, and a light and consistent pressure must be applied to the udder through the transducer to minimize variations in image acquisition (Fasulkov, 2012; Ribeiro *et al*., 2021; Seibt *et al.,* 2023; Themistokleous *et al*., 2023).

Figure 3. Ultrasound evaluation of the size of the udder cistern of the forequarter in cross-section.

Source: Bonelli *et al*. (2020)

Ebtsam *et al*. (2020), used ultrasonography in conjunction with diagnostic methods frequently included in the evaluation of the mammary gland, such as California Mastitis Test (CMT), clinical and histopathological examination. Through the direct contact technique, with the aid of a micro convex transducer, multifrequency (3.5 to 8.5MHz) it was possible to evidence changes in the mammary parenchyma of goats with mastitis, such as changes in the echogenicity of the udder glandular parenchyma and loss of homogeneity of the same, in addition to abscesses in the parenchyma and fibrosis in animals with chronic mastitis. The parenchyma of a mammary gland with heterogeneous echogenicity is demonstrated in figure 4 (Ebtsam *et al*., 2020; Ribeiro *et al*., 2021).

Figure 4. B-mode ultrasonography image demonstrating the presence of parenchymal alteration, linked to the presence of abscess.



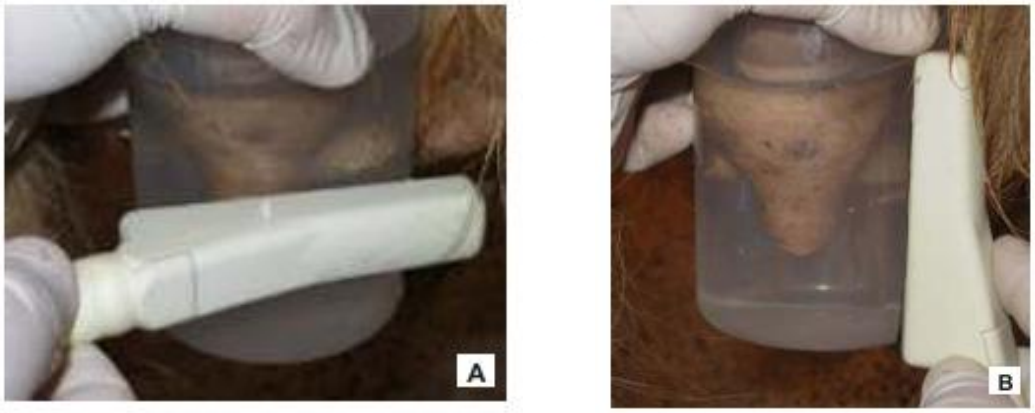
Source: Personal archive, 2025.

For the examination of the structures of the ceilings (ceiling channel, Furstenberg rosette, and roof cistern), a high-frequency probe (7.5 MHz or higher) is used (Gangwar, 2023). During the scan, the probe can be positioned vertically or horizontally in relation to the mammary gland, making it possible to obtain cross-sectional and longitudinal images, respectively (Fasulkov, 2012; Ribeiro *et al*., 2021; Seibt *et al*., 2023).

7 SCANNING BY THE "BAIN-MARIE" TECHNIQUE

When you want to evaluate the ceiling structures more meticulously, the technique of scanning by immersion in a water bath becomes interesting. As described in the literature, in this technique, a 200ml plastic cup filled with water at around 30 to 35 degrees is used, so that the teat is immersed (Tóth *et al*., 2023). To acquire clear images, it is important to perform the exam 30 minutes before milking, use a 7.5 MHz linear transducer, and add the contact gel to the transducer and cup (Ribeiro *et al*., 2021; Abdullah *et al*., 2023). After immersion, the transducer is placed outside the wall of the plastic cup in horizontal and vertical positions, to obtain, respectively, cross-sectional and longitudinal images (Figure 5 A and B) (Ribeiro *et al*., 2021; Tóth *et al*., 2023).

Figure 5. Goat roof ultrasonography by the water bath technique: A – horizontal scan; B – vertical scan.



Source: FASULKOV, 2012.

The water bath technique allows one hand to be used during the examination to manipulate the transducer in different positions along the ceiling, while holding the container with water with the other hand (Ribeiro *et al*., 2021). Through this method it is possible to measure the width of the ceiling, width of the Furstenberg rosette, width of the ceiling cistern, thickness of the ceiling wall, and length of the ceiling canal, as well as the size of the supramammary lymph nodes (Abdullah *et al*., 2023). In addition, typical ceiling walls have three distinct layers: a hyperechogenic outer layer, a thicker and hypoechogenic middle layer, and an inner layer composed of dilated anechoic walls. The clear identification of these layers is essential to differentiate normal tissues from pathological changes, such as edema, inflammation or obstructions, which can modify the echogenicity pattern and indicate mastitis processes or other diseases of the mammary gland (Albino *et al.,* 2017; Amin *et al*., 2017).

Ultrasound is a tool of great value in dairy cattle farming, because, in addition to assisting in reproductive management, it enables the diagnosis of mammary gland pathologies, contributing to the adoption of preventive measures and appropriate treatments. Thus, it is necessary to deepen studies that support the performance of clinical examination and the application of ultrasound techniques.

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