



RESEARCH ARTICLE

Ultrasonographic Examination of Mammary Glands in Lactating Jennies (*Equus asinus*)

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ABSTRACT

The purpose of the present study was to describe the normal ultrasonographic characteristics of the udder and teats in lactating Jennies. Seven lactating Baladi Egyptian Jennies were examined ultrasonographically using 6-8 MHz linear probe. Sagittal and transverse scans of the udder parenchyma and teats were performed for right and left halves through direct (transcutaneous) and indirect (modified stand-off pad) techniques. Measurements of the TCL (teat canal length), TCD (teat canal diameter), TWT (teat wall thickness) and TCD (teat cistern diameter) of both right and left teats were compared for significant difference. The udder parenchyma was identified as coarse granular hypoechoic structure with multiple anechoic cavities. The teat has 3 distinct layers; the outer hyperechoic skin, the inner hyperechoic mucosa and the hypoechoic muscle layers. No significant differences were observed between measurements of the right and the left side. In conclusion, ultrasonography is a safe, effective and non-invasive method that allowed the visualization of the mammary gland (parenchyma and teat) in donkeys. Alteration from the normal ultrasonographic appearance could be helpful for diagnosing pathological conditions of the udder and teats.

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INTRODUCTION

Donkey's milk has successfully been used as a substitute for human milk, especially in children with cow milk-allergy (Monti *et al.*, 2008; Perna *et al.*, 2015). The composition of donkey milk is very close to human milk, especially the lipids content (high levels of lenoleic and lenolenic acid) and proteins (low caseins content). Furthermore, high lysozyme content of donkey's milk may be the cause of its low bacterial count when compared with bovine, caprine and human milk (Vincenzetti *et al.*, 2008).

Ultrasonography is a rapid, safe, effective and non-invasive diagnostic tool used for examination of different organs including the mammary gland and teats (Kahn, 2004; Nishimura *et al.*, 2011). It can be helpful in the diagnosis of mastitis, hematomas, abscesses, tumors, foreign bodies and milk stones (Condino *et al.*, 2012; Santos *et al.*, 2015). Udder and teat scanning is generally performed for the diagnosis of milk flow disturbances and also for examination and measurement of different anatomical structures (Weiss *et al.*, 2004; Paulrud *et al.*, 2005). Ultrasonography had been used for examination of various physiological and pathological conditions of the

mammary gland and teat in cows (Franz *et al.*, 2004; 2009), buffaloes (Kotb *et al.*, 2014), sheep (Rovai *et al.*, 2008; Barbagianni *et al.*, 2015), camels (Abshenas *et al.*, 2007), mares (Güngör *et al.*, 2005) and carnivores (Trasch *et al.*, 2007). Only few available studies have been published describing the ultrasonographic appearance of mammary gland and teat in donkeys (D'Alessandro *et al.*, 2015). The purpose of the present study was to describe the normal ultrasonographic appearance of the mammary gland in lactating Jennies.

MATERIALS AND METHODS

Animals: The present study was performed on 7 clinically healthy multiparous Baladi Egyptian Jennies (*Equus asinus*) aged between 5 and 8 years (mean; 6.3±1.7 years) and weighing between 160 and 230 kg (mean; 190±25 kg). Jennies were evaluated to have body condition score of 6 (more than moderate) and 7 (good) based on a nine-point score system for donkeys (Pearson and Ouassat, 2000). All Jennies were lactating and were examined ultrasonographically during the first month (n=2), second month (n=3) and the third month (n=2) of lactation. All study procedures were done in accordance with

Institutional Animal Care and Use Committee (IACUC) of Faculty of Veterinary Medicine, Cairo University, Egypt.

Before enrollment in the study, all Jennies underwent complete physical and hematological examination (complete blood count and liver and kidney function tests) to exclude the possibility of systemic diseases. The udder was carefully examined for presence of morphological or pathological abnormalities.

Ultrasonographic examination: Ultrasonographic examination of the udder and teats of each animal was performed in standing position using Samsung Madison machine (Sonovet R3, Korea) equipped with 6-8 MHz linear probe. The udder was examined using the direct method (transcutaneous ultrasonography), where the probe was placed directly over the skin after application of coupling gel. The probe was positioned along the length and width of the mammary gland, designated as sagittal and transverse scans, respectively (Güngör *et al.*, 2005). The echo-structures of each mammary half including the mammary parenchyma, the gland cistern and the lactiferous ducts were imaged. The blood vessels (arteries and veins) within the parenchyma were identified and differentiated from the lactiferous duct using color flow Doppler imaging.

The teats were examined by indirect method through using homemade fluid-filled latex bag (modified stand-off pad method) containing warm water placed between the teat and probe (Kotb *et al.*, 2014). The water bag was used to allow localization of the teat at the focal zone of transducer to obtain good quality images. Sagittal and transverse teat scans were recorded. Sagittal scan was obtained by placing the linear probe parallel to the teat, while the transverse scan was obtained by rotating the probe 90°. The echo-structures of the teat were clearly visualized including the papillary orifice, teat canal, Furstenberg's rosette, papillary part of the cistern and the teat wall. The teat canal length (TCL), teat canal width (TCW), teat wall thickness (TWT) and teat cistern diameter (TCD) were measured for both right and left halves (Abshenas *et al.*, 2014).

All examinations were performed by the same examiner and all data was stored digitally and analyzed offline. Measurements were made in triplicates and the average was calculated.

Statistical analysis: Measurements were expressed as mean±SD. Data were analyzed using SPSS software 21 (IBM SPSS Inc., Chicago, IL). Kolmogorov-Smirnov test and Shapiro-Wilk test were used to test the normality of distribution of the obtained measurements. A paired t-test was used to compare measurements of right and left halves. Differences were considered statistically significant if the P value was <0.05.

RESULTS

Transcutaneous ultrasonography of the lactating mammary gland parenchyma in Jennies was easily performed, where the udder was seen to be consisting of two hypoechoic halves separated by hyperechoic septum (Fig. 1). The udder parenchyma was easily identified as

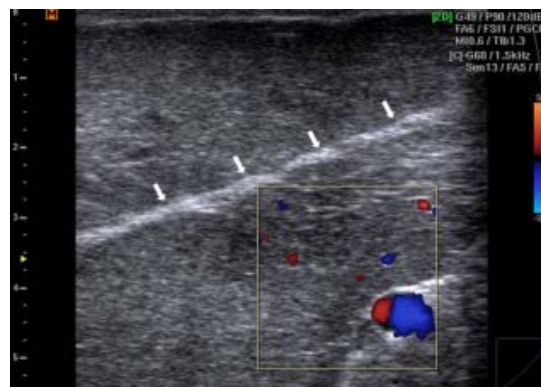


Fig. 1: Sagittal ultrasound scan of udder parenchyma in lactating Jenny demonstrating a homogeneous coarse granular hypoechoic right and left halves separated by hyperechoic septum (arrows).



Fig. 2: Sagittal ultrasound scan of udder parenchyma in lactating Jenny demonstrating homogenous coarse granular hypoechoic udder with multiple anechoic areas representing blood vessels and lactiferous duct (arrows) (A). Color flow Doppler imaging of the same ultrasound scan differentiating blood vessels (arrows) from lactiferous duct (B).

homogenous hypoechoic coarse granular structure. Multiple anechoic zones were identified within the hypoechoic parenchyma, representing the lactiferous ducts and blood vessels. The lactiferous ducts appeared as elongated anechoic branching structure within the hypoechoic parenchyma without echogenic wall. The

blood vessels (arteries and veins) were identified as elongated anechoic branching structures with a characteristic echogenic wall. Color flow Doppler imaging allowed definite differentiation of blood vessels from lactiferous ducts (Fig. 2). Multiple anechoic cisternal cavities were visualized within the udder parenchyma (Fig. 3).

In sagittal scan, the teat was identified as short, cone shaped structure with three distinct layers: A thin outer hyperechoic layer representing the skin (TWT: 2.6 ± 0.2 mm in right half and 2.7 ± 0.1 mm in left half; $P > 0.05$), followed by thick less echogenic homogenous muscular layer and a third thin, hyperechoic mucosal layer (Fig. 4). The teat canal was formed by the mucosal layers of both sides and appeared as a thin hypoechoic line between thicker parallel hyperechoic bands (TCL: 16.3 ± 2.2 mm in right half and 15.5 ± 1.9 mm in left half; TCW: 5.2 ± 0.5 in right half and 5.1 ± 0.4 mm in left half; $P > 0.05$). The teat canal was measured from the inner edges of the mucosa. The teat cistern was identified as a round anechoic structure with hyperechoic wall between the anechoic lactiferous duct and teat canal (TCD: 28.4 ± 3.5 mm in the right half and 29.1 ± 2.7 mm in the left half; $P > 0.05$). The transition between the teat cistern and teat canal is the rosette of Furstenberg which appeared as 3-5 parallel hyperechoic lines. In transverse scan, the teat appeared as ovoid multilayered structure of different echogenicity; the outer hyperechoic skin, the middle hypoechoic muscular layer and the inner hyperechoic mucosa (Fig. 5). No remarkable ultrasonographic differences were recorded in Jennies at different lactation periods (1, 2 and 3 months of lactation). Similarly, differences between measurements obtained for the right and left teats were non-significant.

DISCUSSION

The udder and teat in donkeys are amenable to ultrasonographic imaging because of their superficial location where the visualization of udder parenchyma, lactiferous duct, teat cistern, rosette of Furstenberg, and teat canal was feasible.

Visualization of the udder parenchyma could be achieved through direct transcutaneous ultrasonography while visualization of the teat's structures required placement of a home-made fluid-filled latex bag (modified stand-off pad) between the probe and teat. This may be due to small size and superficial location of teat. Scanning of superficial structures poses a serious problem as the near field reverberations results in image obscuring; so the use of stand-off pad during examination of superficial structures (less than 3.5 cm depth) is highly recommended (Hoque *et al.*, 2004; Rambabu *et al.*, 2008). Moreover; the use of water path increased the acoustic impedance difference between the teat wall and surrounding medium (Kotb *et al.*, 2014).

Similar to other studies, the glandular parenchyma of the udder appeared as homogenous coarse granular hypoechoic structure with presence of multiple anechoic alveoli inside it (D'Alessandro *et al.*, 2015). However, according to Ayadi *et al.* (2003) and Güngör *et al.* (2005), the glandular parenchyma was hyperechoic structure with anechoic alveoli inside it. This variation in the ultrasonographic appearance of udder parenchyma may be



Fig. 3: Sagittal ultrasound scan of udder parenchyma in lactating Jenny demonstrating large cisternal cavities represented by multiple anechoic areas (arrows) within the hyperechoic udder parenchyma.

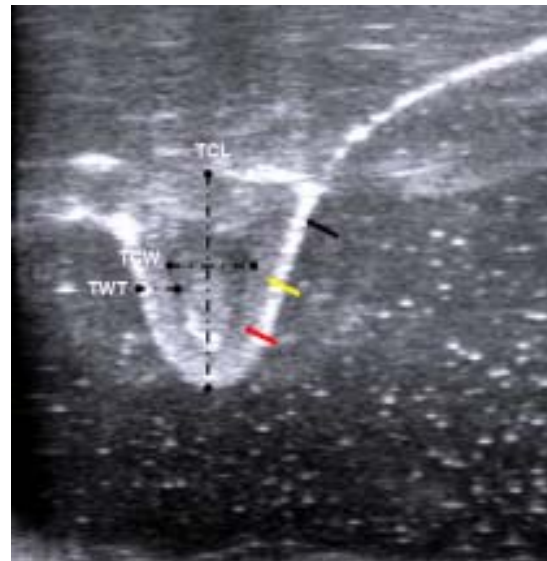


Fig. 4: Sagittal ultrasound scan of the teat in lactating Jenny demonstrating the multi-layered structure of the teat including outer thin hyperechoic skin (black arrow), thick, hypoechoic muscle layer (yellow arrow) and thin hyperechoic mucosa (red arrow). The teat canal length (TCL), teat canal width (TCW) and teat wall thickness (TWT) are also presented.

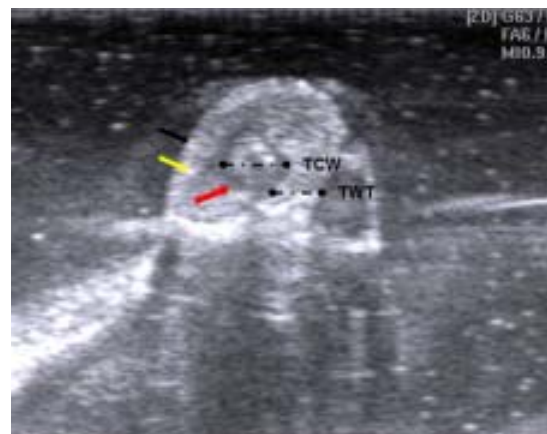


Fig. 5: Transverse ultrasound scan of the teat in lactating Jenny demonstrating the multi-layered structure of the teat including the hyperechoic skin (black arrow), hypoechoic muscle layer (yellow arrow) and hyperechoic mucosa (red arrow). The teat canal width (TCW) and teat wall thickness (TWT) are also presented.

attributed to the variation of the amount of milk within the udder parenchyma, as milk produces acoustic enhancement for the neighboring structures and results in hyperechoic appearance (D'Alessandro *et al.*, 2015). Color flow Doppler imaging of the udder was helpful for differentiating the blood vessels from the lactiferous duct within the udder parenchyma through discoloration of the vessels and pulsation (Götze *et al.*, 2010).

The indirect technique used for imaging the teat in the present study allowed good visualization of the teat structures as well as measuring the teat parameters from both sagittal and transverse scans (Abshenas *et al.*, 2014; Kotb *et al.*, 2014). The presence of milk within the teat also allowed good visualization of the teat canal as milk acts as a window of acoustic impedance difference that allowed imaging of the deeper structures and far wall of the teat (Franz *et al.*, 2003; D'Alessandro *et al.*, 2015).

The main limitation of the present study is the use of relatively low number of lactating Jennies only during the first three months of lactation. Further studies should be directed toward imaging on a large scale in lactating and non-lactating Jennies and the use of ultrasonography in diagnosing pathological udder conditions in Jennies.

Conclusions: ultrasonography is a safe, effective and non-invasive method that allowed the visualization of the mammary gland (parenchyma and teat) in donkeys. Alteration from the normal ultrasonographic appearance could be helpful for diagnosing pathological conditions of the udder and teat that could not be detected by clinical examination.

Author's contribution: All authors conceived and designed the study. FAT did the ultrasonographic examinations; EAH did the ultrasonographic measurements; AIA and AAS analyzed the ultrasound images and measurements. All authors critically reviewed the manuscript for important intellectual contents and approved the final version.

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