



## Study of ultrasound scanning as method to estimate changes in teat thickness due to machine milking in Manchega ewes



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### ABSTRACT

To assess the ultrasound technique as a method for estimating the effect of machine milking on teat thickness in Manchega ewes, this work was carried out with a twofold objective: (1) to study the most suitable ultrasound exploration frequency and the effect of the operator in applying the technique and (2) to validate the approach, comparing it with measurements taken by another method (cutimeter). Three studies were designed for this purpose. In the first of them (E1), two experiments were carried out; the first tested frequencies at 5, 7.5 and 10 MHz and the second (E2) checked frequencies at 3.5 and 5 MHz. In the second study, 2 different operators (OP) independently performed ultrasound scans on the same animals (experiment 3, E3); they chose the best ultrasound exploration image recorded on video (experiment 4, E4) and took measurements in ultrasound images of ewes (experiment 5, E5). Finally, in the third study (experiment 6, E6) we designed an experiment in which ultrasound examinations and measurements by cutimeter were performed in the same animals.

The variables measured in the ultrasound scans were teat wall thickness (TWT), teat wall area (TWA) and teat end wall area (TEWA), while the cutimeter was used to gauge teat wall thickness (TT). At 10 MHz frequency it was not possible to obtain quality images of teats in ewes, so its subsequent use was ruled out. The teat canal was visible in 45%, 55% and 30% of the pre-milking images at frequencies of 5, 7.5 and 3.5 MHz, with values increasing after milking (63.4%, 77.5% and 36.3%). No significant differences were found in any of the variables (TWT, TWA and TEWA) between the measurements taken before and after milking at the 5 and 7.5 MHz frequencies. Similarly, TWT, TWA and TEWA were significantly greater ( $p < 0.001$ ) at the 3.5 MHz frequency than at 5 MHz. It was concluded that frequencies of 5 and 7.5 MHz were the most suitable for estimating the teat wall changes caused by mechanical milking.

Operator experience had no effect on the ultrasound explorations performed in the field, although it did significantly influence the choice and measurement of the images.

Mechanical milking caused an increase in TWT of 0.11 cm measured by ultrasound scanning and a reduction in TT of  $-0.06$  cm measured by cutimeter, which would indicate that ultrasound scanning is a more effective method to determine the degree of oedema and congestion caused by machine milking in ewes. In addition, we found a high correlation between TWT, TWA and TEWA with TT before and after milking.

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The use of ultrasound techniques may be a useful tool to evaluate the changes that occur in teat tissue due to machine milking in Manchega breed sheep, although it would be necessary to carry out further studies to enable the development of software to choose and measure the frames automatically to help reduce the operator effect on the choice and measurement of ultrasound variables.

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## 1. Introduction

Machine milking can cause an alteration in the teat tissue fluid dynamics and a consequent negative effect on the teat's defence mechanisms (Paulrud et al., 2005) that may lead to higher intramammary infection rates (O'Shea, 1987). To estimate the changes taking place in the teat end and teat wall, different qualitative methods have been proposed, such as changes in teat colour (Hillerton et al., 2002) or the degree of oedema and congestion at the base and end of the teat (Mein et al., 2001), as well as quantitative approaches such as laser Doppler flowmetry (Persson, 1991), infrared thermography (Paulrud et al., 2005), cutimeter (Hamann et al., 1996) and ultrasound (Neijenhuis et al., 2001).

Different studies carried out using ultrasound techniques have shown that mechanical milking, even when performed in suitable conditions, causes an increase in thickness of the teat wall (TWT) in cows (Gleeson et al., 2002; Neijenhuis et al., 2001; Paulrud et al., 2005), goats (Fahr et al., 2001; Ślósarz et al., 2010) and sheep (Wójtowski et al., 2006). When teat thickness was measured by cutimeter, an increase was also reported in cows (Forsbäck et al., 2005; Hamann and Mein, 1990; Tamburini et al., 2010) and goats (Fahr et al., 2001; Manzur et al., 2012; Skapetas et al., 2008). However, in sheep the teat thickness measured by cutimeter after machine milking was reduced (Peris et al., 2003a), even when overmilking took place (Peris et al., 2003b). The differences in the change in thickness of teat tissues caused by machine milking may be due to the fact that with ultrasound scanning no pressure is exerted on the teat, whereas with the cutimeter the jaws exert a pressure that forces re-evacuation of the blood, reducing the congestion that may have been caused (Hamann and Mein, 1990).

In recent years, ultrasound has been used in sheep to study the internal teat structures (Franz et al., 2001), the relation between teat anatomy and intramammary infection (Franz et al., 2003) and the changes in teat tissue due to machine milking (Wójtowski et al., 2006).

In goats, exploration frequencies of 5 MHz (Díaz et al., 2013; Fasulkov et al., 2010), 7.5 MHz (Díaz et al., 2013) and 10 MHz (Díaz et al., 2013; Fahr et al., 2001; Ślósarz et al., 2010) have all been used to estimate the variation in thickness of the teat wall caused by machine milking, although Díaz et al., 2013 do not recommend the use of the latter. However, in sheep there are few studies on the use of ultrasound scanning to study teat tissues. Wójtowski et al., 2006 used a 10 MHz frequency to determine the effects of machine milking on teat wall thickness, whereas frequencies of 5 and 8.5 MHz (Bruckmaier and Blum, 1992; Franz et al., 2003, respectively) have been used to study the

internal teat structures. One of the factors to be considered when applying the technique is the operator's experience in capturing and measuring the ultrasound images, since in studies carried out in cows (Klein et al., 2005) and goats (Díaz et al., 2013; Ślósarz et al., 2010) it was observed that the experience of the operator can have a significant effect on the measurements taken.

As there is little information on the use of ultrasound technique to estimate changes in teat tissue in Manchega breed sheep, the present work was proposed with a dual objective: (1) to study the most suitable ultrasound exploration frequency and the effect of the operator in applying the technique; (2) to validate the approach, comparing it with measurements taken by another mediation method (cutimeter).

## 2. Materials and methods

### 2.1. Animals, housing and management

The experiments were carried out at the Educational and Research Farm of the Escuela Politécnica Superior de Orihuela (EPSO) belonging to Miguel Hernández University (UMH) and the Experimental Farm of the Animal Science Technology Institute (ICTA) at the Universidad Politécnica de Valencia (UPV).

The UMH farm herd consists of 80 Manchega breed ewes. During experiments, animals were kept in free stall housing, with daytime access to exercise yards. The milking parlour was "Casse" type with a quick exit stalls, one platform with 12 places and 12 low-line milking units (1 × 12 × 12). The facility had electronic milk recorders and teatcups with automatic vacuum shut-off valves and silicone liners (Top Flow S) (Gea Farm Technologies, Bönen, Germany). The milking parameters used were 36 kPa vacuum level (VL); 180 ppm pulsations rate (PR) and 50% pulsation ratio (PRT).

The ICTA Small Ruminants Experimental Farm of the UPV has capacity for 90 Manchega breed ewes. During the experiments, the animals were housed in permanent stabling conditions. The milking parlour was "Casse" type with a quick release trap and automatic feeder trough, 2 platforms with 12 places each and six midline milking units (2 × 12 × 6). The milking unit consisted of a collector (SG-TF80 ML) with manual shut-off key and the teatcup used had automatic shut-off valve and silicone liner (Almatic S10) (DeLaval International AB, Tumba, Sweden). The milking parameters used were: VL: 36 kPa, PR: 180 ppm, PRT 50%.

On both farms, 5 weeks after parturition the lambs were separated from the ewes, which were then machine milked twice daily. In addition, the machine milking routine for the animals consisted of teatcup placing, mechanical milking, machine stripping and teatcup removal. Finally, after milking teats were immersed in an iodine solution.

### 2.2. Experimental design and data processing

To achieve the stated aims, three studies were carried out:

- Exploration frequencies study.
- Study of operator effect in application of the technique.
- Method validation study: comparison with cutimeter.

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