

# The Influence of Milking on the Teat Canal of Dairy Cows Determined by Ultrasonographic Measurements

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## ABSTRACT

Strapák P., Strapáková E., Rušinová M., Szencziová I. (2017): **The influence of milking on the teat canal of dairy cows determined by ultrasonographic measurements.** Czech J. Anim. Sci., 62, 75–81.

The effect of milking on the length and diameter of the teat canal during teat regeneration immediately after milking was evaluated. The practical measurements of the teat canal were performed on 70 Holstein cows using ultrasound Aloka Prosound device 2. A special “bath method” of the teat ultrasonographic scanning was used at set time intervals – before milking, immediately after milking, and 30, 60, 90, and 120 min after milking. Before milking, the average length of the teat canal was 10.67 mm and 9.3 mm and the teat canal diameter was 1.11 mm and 1.09 mm for front and rear teats, respectively. The average length of the front and rear teat canal significantly increased by about 20.5 and 32.9% and the front and rear teat canal diameter increased by 9.0 and 9.1% on average immediately after milking compared to values detected before milking. After 120 min of regeneration the teat canal was by 3.56 and 14.95% longer than before milking for front and rear teats, respectively. Prolongation of the teat canal was significantly affected by teat position. Based on the present results, the time period of 120 min is not sufficient for the complete regeneration of the teat canal length to the pre-milking values. On the other hand, we recorded total regeneration of the teat diameter within 120 min after milking. The initial values of the front and rear teat diameter were equal to those measured 120 min after milking.

**Keywords:** ultrasonography; udder; teat regeneration; milking traits

In recent years, new techniques are being used on a larger scale in livestock production. Some modern diagnostic procedures are referred to as imaging diagnostic methods. A very successful method is ultrasonography, which is employed in veterinary practice in the diagnosis of early-pregnancy, the

examination of genital organs of dairy cows, and also for purposes of diseases diagnostics. Hospes and Seeh (1999) pointed out the non-invasivity of ultrasonography, with the possibility of monitoring the structures of mammary glands, teats, and parenchyma. Ultrasonography has an important

Supported by the Cultural and Educational Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic (KEGA) (Project No. 035SPU-4/2015) and by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences (VEGA) (Project No. 1/0724/16).

role in evaluation of the udder health status (Braun 1997) and does not only visualize the ridge of the milk cistern and the teat canal, but also the anatomical structures located in their proximity (Geishauser and Querengasser 2000; Fasulkov 2012). The structure and the anatomy of the udder are important for milk production and machine milkability (Ayadi et al. 2003).

The mammary gland comes into contact with many infectious microorganisms that may penetrate through the teat canal into the internal environment of the udder and can cause inflammation. Paulrud (2005) reported that the bovine teat canal has unique function of preventing leakage of milk and entry of bacteria. Teat canal epithelia are derived from the primary germ cell layer called ectoderm, just like the cells skin and the function this unique biological membrane includes is the role of sealant of the teat canal during the dry period and between milkings. Teat canal keratin physically blocks the teat canal and in this way prevents penetration of potential mastitis-causing bacteria. The bovine teat canal has a structure of stratified squamous epithelium in which the superficial strata undergo keratinization and cellular detritus is eliminated during milking through the teat canal.

In the time interval between two milkings the teat canal is closed with the teat sphincter, which is considered as the first physical defense system against the penetration of microorganisms into the udder (Strapak et al. 2013). Seyfried (1992) noted that a teat canal that is too short or too long increases the risk of bacterial colonization in the udder. Scherzer (1992) stated a nonsignificant relationship between the occurrence of acute mastitis and the teat canal length.

During milking the teat orifice opens, and thus suitable conditions for the penetration of microorganisms into the udder are created. The teat canal remains open for a few hours after the end of milking. The time period in which the teat canal returns to the pre-milking state depends on many internal and external factors such as the functional parameters of milking machine, the milking technique, the age of the animals, breed, production type (Tancin and Tancinova 2008), the shape of the teats (Rathore 1977; Tilki et al. 2005), as well as characteristics of the teats (Gleeson et al. 2005; Paulrud et al. 2005; Stadnik et al. 2010, and others).

The influence of milking on changes in teat characteristics was investigated by Neijenhuis et al. (2001) in Holstein cows using ultrasonography. They evaluated the main characteristics of the teats before milking, immediately after milking, and during regeneration of the teats every hour for eight hours after the end of milking. The authors reported that the regeneration of the teat characteristics occurred 240–480 min after the end of milking, which was however significantly affected by the individuality of cow.

The influence of milking on the length and diameter of the teat canal in Holstein cows was also analyzed by Stadnik et al. (2010), who found that the recovery of the teats was still not complete even 180 min after the end of milking. Similar findings have also been stated by Stojnovic and Alagic (2012), who confirmed the increased length of the front and rear teat canal influenced by milking.

Grindal and Hillerton (1991) found out that the width in the teat canal is a positively correlated parameter, which means that with increasing width of the teat canal the risk of infection increases. On the contrary, the teat canal length is estimated as a negative correlated parameter which means that the longer the teat canal, the smaller the risk of penetration of the pathogen into the mammary gland below.

The aim of the present study was to measure the length and the diameter of the teat canal using ultrasonographic scanning before and at several time intervals after milking and to assess morphological changes in teats of cows, occurring as a reaction to mechanical milking.

## MATERIAL AND METHODS

The experiment was carried out at an experimental farm in Slovakia. A total of 70 Holstein dairy cows in the first, second, or third and higher parity were included in the data set.

Milking was performed in a 2 × 10 herringbone milking parlor (BouMatic, USA) at 60 pulses/min, a vacuum level of 42 kPa, and pulsation rate for front teats of 61 : 39 and for rear teats of 63 : 37. An automatic cluster take-off system at critical milk flow rate 0.2 kg/min was used.

Ultrasound images of the longitudinal cross-section of the teats were taken at morning milking in regular time intervals, i.e. before milking (after

doi: 10.17221/68/2015-CJAS

udder preparation) and 0, 30, 60, 90, 120 min after milking. Measurements were performed on the right front and right rear teats. The experiment was carried out on 140 teats with a total of 840 teat measurements taken.

The measurements of internal traits of the teats were performed by an ultrasound device Prosound 2 with a linear probe UST-586 (both Hitachi Aloka Medical, Ltd., Japan) with the walking frequency of 7.5 MHz recommended for teat structure measurements by Franz et al. (2001) and Fasulkov (2012). Teat measurements were taken using a special method called “water bath method”, when a plastic container filled with warm water (38°C) and a built-in linear probe were used. The teats were immersed into the water bath and the ultrasound images were stored in a storage medium. According to Franz et al. (2001), one of the major advantages of using the water bath method is the chance to manipulate the probe in a vertical position along with the movement of the plastic container. The use of this teat measuring method prevents deformations of the teat, which may occur when the direct contact method is used.

The ultrasonographic images were evaluated and analyzed using the NIS 3.2 software. From each image, the length and the diameter of the teat canal were determined. The teat canal length was measured in millimeters as the distance between the distal and proximal end (accuracy of 1 mm) and the diameter of the teat was taken in the middle of the teat canal (Figure 1).

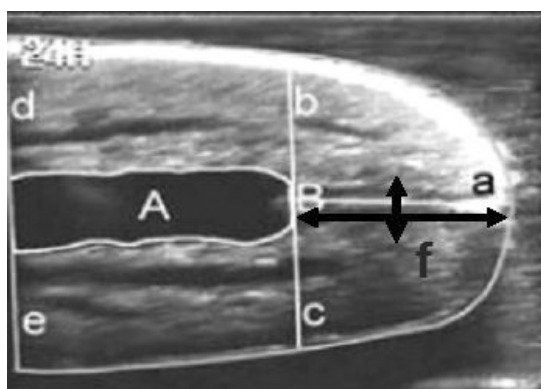


Figure 1. Ultrasound image of internal parameters of a teat (Huth 2004)

A = teat cistern; B = rosette of Furstenberg; a = teat canal length; b, c = teat wall thickness at Furstenberg's rosette; d, e = teat wall thickness 10 mm from Furstenberg's rosette; f = teat canal diameter (in the middle of the teat canal)

The basic statistical parameters were calculated for all the observed traits prior to and at 0, 30, 60, 90, and 120 min after milking. Analyses of variance and Tukey's studentized range test (HSD) were used to compare the absolute changes of the internal traits of teats – the length and the diameter of the teat canal before milking, and at 0, 30, 60, 90, 120 min after milking. *P*-level for statistical significance was  $P < 0.05$ . The data were analyzed by ANOVA through the SAS software (Statistical Analysis System, Version 9.2, 2008).

A linear model with fixed effects parity, lactation stage, teat position, and recovery time was used to identify the main sources of variation in the length and diameter of teats. Least Squares Means ( $\mu + \alpha$ ) and standard errors (SE) were calculated for all observed traits.

The following equation was used:

$$Y_{ijklm} = \mu + \text{parity}_i + \text{stage}_j + \text{position}_k + \text{recovery time}_l + e_{ijklm}$$

where:

$Y_{ijklm}$  = dependent variable (length and diameter of teat canal)

$\mu$  = average value of dependent variable

$\text{parity}_i$  = fixed effect of the  $i^{\text{th}}$  lactation ( $i = 3$ )

$\text{stage}_j$  = fixed effect of the  $j^{\text{th}}$  stage of lactation ( $j = 3$ )

$\text{position}_k$  = fixed effect of the  $k^{\text{th}}$  teat position (front, rear)

$\text{recovery time}_l$  = fixed effect of the  $l^{\text{th}}$  time period ( $l = 6$ )

$e_{ijklm}$  = residual effect

## RESULTS AND DISCUSSION

Before milking, the average length of the front teats was 10.67 mm and of the rear teats 9.36 mm (Table 1). Similar findings were reported e.g. by Grindal and Hillerton (1991), Hamann et al. (1994), and Geidel and Graff (2001). The average diameter of the teat canal reached 1.11 mm in the front teats, and 1.09 mm in the rear teats (Table 1). A higher average value of the teat canal diameter in Holstein cows (all teats of each cow) – 1.7 mm – was stated by Klein et al. (2005).

Milking led to prolongation of the teat canal to 12.86 mm after the end of milking, representing an elongation of 20.5% on average. The rear teats prolonged to 12.44 mm at the end of milking, representing an elongation of 32.9% (Table 1,

Table 1. Influence of milking on the teat canal length and diameter in Holstein cows ( $n = 70$ , values are means  $\pm$  standard deviation)

Factor	Time of measurement					
	before milking	after milking	30 min after milking	60 min after milking	90 min after milking	120 min after milking
<b>Front teat</b>						
Teat canal length	10.67 $\pm$ 1.53 <sup>e</sup>	12.86 $\pm$ 1.52 <sup>a</sup>	12.42 $\pm$ 1.46 <sup>a,b</sup>	12.05 $\pm$ 1.42 <sup>b,c</sup>	11.59 $\pm$ 1.43 <sup>c,d</sup>	11.05 $\pm$ 1.43 <sup>d,e</sup>
Teat canal diameter	1.11 $\pm$ 0.10 <sup>c</sup>	1.21 $\pm$ 0.12 <sup>a</sup>	1.18 $\pm$ 0.11 <sup>a,b</sup>	1.16 $\pm$ 0.10 <sup>a-c</sup>	1.14 $\pm$ 0.10 <sup>b,c</sup>	1.11 $\pm$ 0.10 <sup>c</sup>
<b>Rear teat</b>						
Teat canal length	9.36 $\pm$ 1.79 <sup>d</sup>	12.44 $\pm$ 1.77 <sup>a</sup>	12.10 $\pm$ 1.68 <sup>a,b</sup>	11.79 $\pm$ 1.65 <sup>a,b</sup>	11.34 $\pm$ 1.58 <sup>b,c</sup>	10.76 $\pm$ 1.67 <sup>c</sup>
Teat canal diameter	1.09 $\pm$ 0.09 <sup>c</sup>	1.19 $\pm$ 0.10 <sup>a</sup>	1.16 $\pm$ 0.09 <sup>a,b</sup>	1.15 $\pm$ 0.08 <sup>a,b</sup>	1.12 $\pm$ 0.08 <sup>b,c</sup>	1.10 $\pm$ 0.08 <sup>c</sup>

$P < 0.05$ , means with the same letter are not significantly different

Figure 2). These values seem higher compared to results of Geidel and Graff (2001) who found extension of front teats of 17% on average and rear teats of about 24%. Based on the measurements in Holstein dairy cows, Szencziová et al. (2013) reported an average enlargement of the teat canal by 2.41 mm (22%). Similar conclusions were also made by Stadnik et al. (2010), who confirmed the extension of the front teat canal in Holstein dairy cows due to milking by 13.9%. Stojanović and Alagić (2012) reported that due to milking the front teats extended by 1.7 mm (15.44%) and the rear teats by 2.59 mm (24.38%) on average. Greater differences in the changes of the teat canal length due to milking were reported by Celik et

al. (2008) (20–40%) and by Paulrud et al. (2005) (30–41%). These outcomes can be explained by the use of different kinds of measuring methods and mechanical manipulation with the teats, or by different time of examination, parity, and genetic differences between the breeds.

In this study the impact of milking on the teat canal diameter was also analyzed. The front teats canal diameter increased from 1.11 mm before milking to 1.21 mm after the end of milking (9%) while the rear teats canal diameter from 1.09 mm before milking to 1.19 mm after milking (9.1%) (Table 1, Figure 3). Szencziová et al. (2013) stated an increase in the average diameter of the teat canal by about 17%. Higher value may be caused

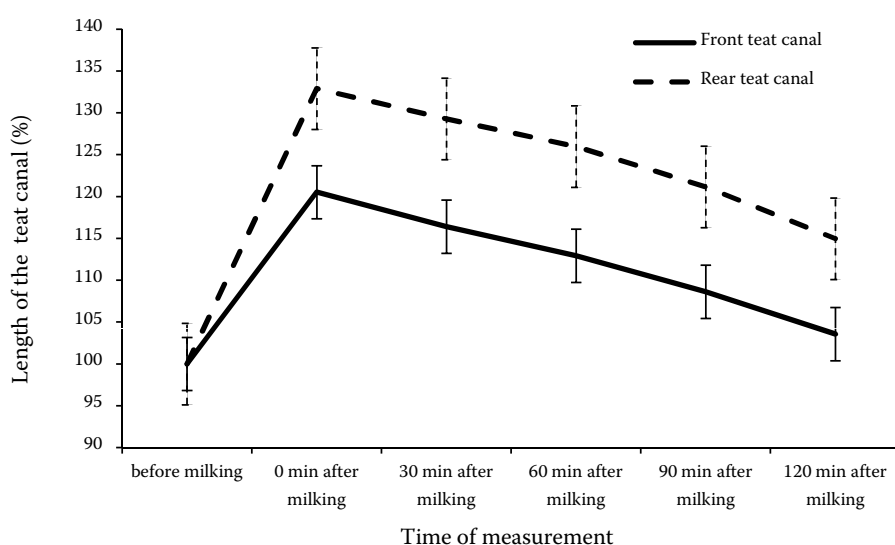


Figure 2. Influence of milking on relative changes of the teat canal length with standard errors

doi: 10.17221/68/2015-CJAS

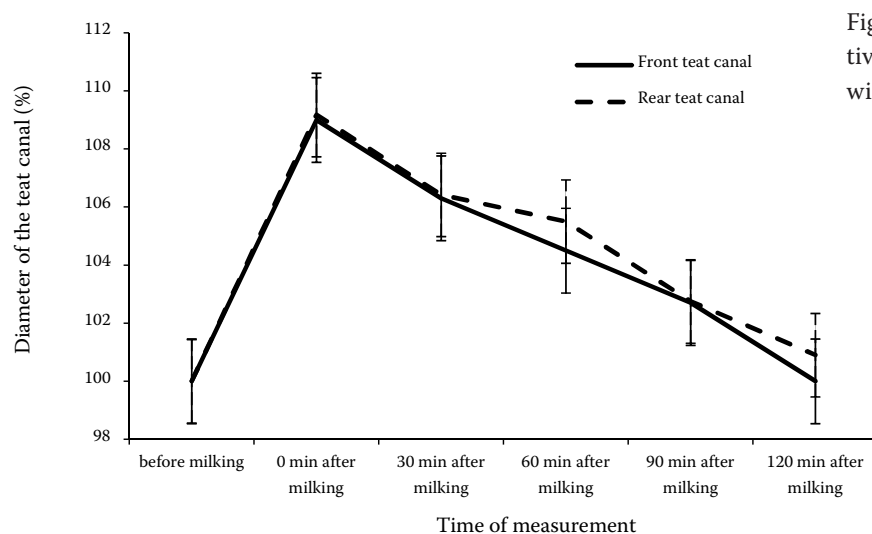


Figure 3. Influence of milking on relative changes of the teat canal diameter with standard errors

by a low number of cows in experiment ( $n = 25$ ), parity, or stage of lactation. In contrary to our results, Fasulkov et al. (2014) showed that a teat canal diameter was reduced by 9.89% immediately after milking. The authors supposed that this could be attributed to the effect of vacuum and mechanical influence of milking on teat tissues.

The process of regeneration of the teat canal length and diameter is displayed in Table 1. Means marked with the same letter were not significantly different. The length and diameter of the teat canal was reducing, confirming the progressive regeneration process of teats after the end of milking. It indicated that the teat canal length regeneration in dairy cows had a straightened tendency to decline (Table 1, Figure 2). The results showed significant differences between most of measured average values of the teat canal length and diameter during teat regeneration (after milking to the values of these traits in the idle state – before milking). This study confirmed that the values of the teat canal length after a 120 min recovery compared to those before milking achieved 103.56% for the front teats and 114.95% for the rear teats. The initial diameter of the front and rear teats was 1.11 and 1.09 mm, respectively. These measurements were comparable with values taken 120 min after milking (1.11 and 1.10 mm) (Table 1). Based on these results the time period of 120 min was not enough for the teat canal length regeneration in Holstein dairy cows but the diameter of the teat canal was almost fully regenerated.

The teat tissue regeneration after milking is a quite lengthy and continuous process, which is

generally characterized as the time required for closing. Enclosed teat reduces the penetrability of the microorganism endotoxin into the teat canal after milking (McDonald 1975). Based on the previous studies, the minimum time for the teats recovery is 120–240 min after the end of milking. Stadnik et al. (2010) evaluated the time period required for the recovery of the teat canal in Holstein dairy cattle, and found that the difference in the value after 180 min after the end of milking was 0.04 mm (+2.96%), suggesting that 180 min after the end of milking the teat canal is still not recovered.

McDonald (1975) and Neijenhuis et al. (2001) stated that even after 360 and/or 480 min after the end of milking the regeneration of the teat canal is not fully completed. For these reasons, they recommended that with the help of modern technology the dairy cows should be prevented from lying down for at least 120 min after the end of milking.

In contrast, Huth (2004) found no differences when evaluating the changes of length and diameter of the teat canal in Hungarian Simmental dairy cows 120 min after the end of milking, and stated their complete regeneration. Hamman et al. (1994) pointed out that the evaluation of teat recovery is very important for determining the time period between milkings, because there is a relatively high risk of mastitis due to the unclosed teat canal. However, recent research has shown that the time interval for teats regeneration after milking may be also significantly longer than the above-mentioned times (Neijenhuis et al. 2001).



Table 2. Effect of parity, stage of lactation, teat position, and recovery time on the teat canal length and diameter

Factor	Teat canal length			Teat canal diameter		
	Mean Square	F-value	P > F	Mean Square	F-value	P > F
Parity	11.8563	4.94	0.0074	0.0409	4.94	0.0073
Stage of lactation	33.5296	13.97	< 0.0001	0.4056	48.92	< 0.0001
Teat position	53.6765	22.36	< 0.0001	0.0650	7.84	0.0052
Recovery time	128.8777	53.70	< 0.0001	0.1860	22.43	< 0.0001

$P < 0.05$

Table 2 shows a significant influence of parity, stage of lactation, teat position (front and rear teat), and recovery time on the length and diameter of the teat canal.

Least Squares Means with standard errors of all factors for length and diameter of the teat canal are given in Table 3.

Neijenhuis et al. (2001) used random regression model to determine the factors influencing teat tissue parameters. The authors also found significant effect of recovery time on all evaluated teat traits. Teat position had a significant effect only on the teat-cistern width. Physical dimensions of teat tissues are only one factor, more important may be the dynamic responses of teats.

## CONCLUSION

When analyzing the impact of milking on internal traits of teats caused by milking, the average length of the teat canal for front and rear teats increased by about 20.5% and 32.9%, respectively. The effect of the teat position on elongation of the teat canal immediately after milking was significant. Similarly, the influence of milking on the teat canal diameter extension by 9.0 and 9.1% on average, in the front and rear teats, respectively, was found. Based on the 120-minute period of measurements it was verified that the established traits, the length and the diameter of the teat canal, were decreasing over time, pointing to the ongoing regeneration process

Table 3. Least Squares (LS) and standard errors (SE) of parity, stage of lactation, teat position, and recovery time for length and diameter of teat canal

Parameter	Teat canal length				Teat canal diameter			
	LS	SE	t-value	P >  t	LS	SE	t-value	P >  t
Intercept	9.8684	0.1885	52.35	< 0.0001	1.0371	0.0110	93.60	< 0.0001
Parity 1	0.4098	0.1421	2.88	0.0040	0.0218	0.0083	2.62	0.0090
Parity 2	0.3983	0.1436	2.77	0.0057	0.0252	0.0084	2.99	0.0029
Parity 3	0.0000				0.0000			
Stage 1	0.5740	0.1327	4.33	< 0.0001	0.0770	0.0078	9.88	< 0.0001
Stage 2	0.7171	0.1445	4.96	< 0.0001	0.0516	0.0084	6.08	< 0.0001
Stage 3	0.0000				0.0000			
TPF	0.5055	0.1069	4.73	< 0.0001	0.0175	0.0062	2.80	0.0052
TPR	0.0000				0.0000			
RT 0	−0.8874	0.1851	−4.79	< 0.0001	−0.0062	0.0108	−0.57	0.5682
RT 1	1.74407	0.1851	9.42	< 0.0001	0.0869	0.0108	7.99	< 0.0001
RT 2	1.3437	0.1851	7.26	< 0.0001	0.0625	0.0108	5.75	< 0.0001
RT 3	1.0144	0.1851	5.48	< 0.0001	0.0427	0.0108	3.93	< 0.0001
RT 4	0.5602	0.1851	3.03	0.0026	0.0207	0.0108	1.90	0.0574
RT 5	0.0000				0.0000			

stage = stage of lactation, TPF = teat position – front, TPR = teat position – rear, RT = recovery time

$P < 0.05$

doi: 10.17221/68/2015-CJAS

of the teats after the end of milking. However, based on the present results it may be concluded that the time period of 120 min is not sufficient for the complete regeneration of the teat canal length to the values taken before milking. On the other hand, 120 min after the end of milking, the diameter of the teat canal was almost fully recovered.

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Received: 2015–09–25

Accepted after corrections: 2016–10–06