Short and long-term effects on the teat tissue in a Cow Calf Contact system with automatic milking

Effekter på spenvävnaden i ett ko-kalvsystem med automatisk mjölkning

Johanna Lilja

Degree project • 30 hp
Swedish University of Agricultural Sciences
Department of Animal Nutrition and Management
Agriculture programme – Animal Science
Uppsala 2021

Short and long-term effects on the teat tissue in a Cow Calf Contact system with automatic milking

Effekter på spenvävnaden i ett ko-kalvsystem med automatisk mjölkning

Johanna Lilja

Supervisor:	**Josef Dahlberg, Swedish University of Agricultural Sciences, Department of Clinical Sciences**
Assistant supervisor:	Hanna Eriksson, Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management
Examiner:	Sigrid Agenäs, Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management

Credits:	30 hp
Level:	Advanced, A2E
Course title:	Independent project in Animal Science, A2E - Agriculture Programme - Animal Science
Course code:	EX0872
Programme/education:	Agriculture programme – Animal Science
Course coordinating dept:	Department of Animal Breeding and Genetics
Place of publication:	Uppsala
Year of publication:	2021
Cover picture:	Johanna Lilja

Keywords: Teat end callosity, hyperkeratosis, teat wall thickness, ultrasound, suckling, nursing

Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal Science
Department of Animal Nutrition and Management

Publishing and archiving

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. If you check the box for **YES**, the full text (pdf file) and metadata will be visible and searchable online. If you check the box for **NO**, only the metadata and the abstract will be visible and searchable online. Nevertheless, when the document is uploaded it will still be archived as a digital file.

If you are more than one author you all need to agree on a decision. You can find more information about publishing and archiving here: https://www.slu.se/en/subweb/library/publish-and-analyse/register-and-publish/agreement-for-publishing/

☒ YES, I/we hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work.

☐ NO, I/we do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.

Abstract

The interest in keeping calves with their lactating dams in dairy production is increasing, among both dairy farmers and consumers. This MSc thesis is a part of an ongoing project at the Swedish University of Agriculture (SLU) where a cow-calf contact system with automatic milking is studied. One of the concerns of integrating calves in dairy production has been that the udder health might be impaired, and that the combination of milking and suckling involves a high strain on the teats. Teat end callosity (TEC) is a long-term effect of wearing of the teat caused by machine milking. The swelling of the teat wall during machine milking is a short-term effect on the teat tissue, also caused by machine milking. Both TEC and increased teat wall thickness (TWT) during machine milking might be risk factors for impaired udder health. The purpose of this thesis was to examine differences in TEC and TWT between nursing dairy cows (treatment) and control cows.

The cow-calf project at SLU is divided into several batches. The present study included batch 1 and 2, with a total of 30 treatment cows and 26 control cows housed in a loose house system. Calves had free access to their dams in a contact area and cows were milked in an automatic milking system (DeLaval VMS^{TM} Classic). Calves and cows were separated approximately 16 weeks postpartum. The scoring of TEC was performed according to the scheme developed by Neijenhuis et al. (2000). TEC was scored at four occasions: prior to parturition, about eight weeks postpartum, prior to separation and eight weeks after separation. Any wounds on the teats were noted at each scoring event. TWT was assessed pre- and post-milking by ultrasound about 11 weeks postpartum. 180 images of 90 teats was used for further statistical analysis.

Treatment cows had significantly ($p < 0.05$) lower mean TEC scores post-partum in batch 1 compared to control cows. There were no significant differences in mean TEC score between treatment cows and control cows at the other scoring events. In batch 2 there were no significant differences in mean TEC score between treatment cows and control cows. A total of nine wounds was noted during the whole study period. There was a significant increase ($p < 0.001$) in TWT during milking in both groups, yet, there were no significant difference in TWT increase between treatment cows and control cows.

This study found no evidence that the degree of TEC, the number of wounds, or the TWT were negatively affected by suckling calves in this automatic milking system.

Keywords: Teat end callosity, hyperkeratosis, teat wall thickness, ultrasound, suckling, nursing.

Sammanfattning

Intresset för att hålla kalvar med sina lakterande mödrar i system för mjölkproduktion ökar, både bland mjölkproducenter och konsumenter. Denna masteruppsats är en del av ett pågående projekt vid Sveriges lantbruksuniversitet (SLU) där ett ko-kalvsystem med automatisk mjölkning studeras. Det finns en oro att juverhälsan ska försämras och slitaget på spenarna öka om kalvar hålls tillsammans med mjölkkor. Hyperkeratos på spenspetsarna är en långvarig effekt av slitage på spenen orsakad av maskinmjölkning. Förtjockad spenvägg är en kortvarig effekt på spenvävnaden som kan uppstå under maskinmjölkning. Både hyperkeratos och svullen spenvägg kan vara riskfaktorer för försämrad juverhälsa. Syftet med den här studien var att undersöka skillnader i hyperkeratos på spenspetsarna och tjockleken på spenväggen mellan digivande mjölkkor och kontrollkor.

Ko-kalvprojektet på SLU är uppdelat i flera omgångar. Den aktuella studien inkluderade omgång 1 och 2 med totalt 30 digivande kor och 26 kontrollkor i ett lösgående system. Kalvarna hade möjlighet att dia fritt och korna mjölkades av en mjölkningsrobot (DeLaval VMS™ Classic). Kalvarna avvandes vid ungefär 16 veckors ålder. Bedömningen av hyperkeratos på spenspetsarna utfördes enligt schemat utvecklat av Neijenhuis et al. (2000). Graden av hyperkeratos på spenspetsarna bedömdes vid fyra tillfällen: före kalvning, cirka åtta veckor efter kalvning, före avvänjning och cirka åtta veckor efter avvänjning. Eventuella sår på spenarna noterades vid varje tillfälle. Tjockleken på spenväggarna före och efter mjölkning mättes med ultraljud cirka 11 veckor efter kalvning i omgång 2. 180 bilder av 90 spenar användes för statistisk analys.

De digivande korna hade signifikant (p <0,05) lägre grad hyperkeratos efter kalvning i batch 1 jämfört med kontrollkorna. Det fanns inga signifikanta skillnader i graden av hyperkeratos mellan digivande kor och kontrollkor vid de andra tillfällena för bedömning. I batch 2 fanns inga signifikanta skillnader vad gäller graden av hyperkeratos mellan digivande kor och kontrollkor. Totalt nio sår noterades under hela studien. Det var en signifikant ökning (p <0,001) av spentjockleken under mjölkning i båda grupperna, men det fanns ingen signifikant skillnad i ökning mellan digivande kor och kontrollkor.

Denna studie fann inga bevis för att graden av hyperkeratos, antalet sår eller förändringen av spentjockleken blev negativt påverkad av diande kalvar i det här automatiska mjölkningssystemet.

Nyckelord: Hyperkeratos, spenväggstjocklek, ultraljud, dia, digivning.

Table of contents

Abbreviations

CMS	Conventional Milking System
CMT	California Mastitis Test
DIM	Days in Milk
IMI	Intramammary Infection
IMP	Intramammary Pressure
SCC	Somatic Cell Count
SD	Standard Deviation
SLU	Swedish University of Agriculture
TCL	Teat Canal Length
TD	Teat Diameter
TEC	Teat End Callosity
TEW	Teat End Width
TWT	Teat Wall Thickness
VMS	Voluntary Milking System

1. Introduction

At today's high producing dairy farms, the new-born calves are commonly removed from their dams after consuming colostrum. However, there is an increasing interest among milk producers and consumers in keeping calves together with their lactating dams. This thesis is a part of an ongoing project at the Swedish University of Agriculture (SLU) where a dairy cow-calf contact system with automatic milking is studied.

There are various ways of keeping calves with their lactating dams. Many studies are performed in part-time cow-calf contact systems (Rasmussen & Larsen 1998; Fröberg et al. 2007; Barth 2020), in which the calf have contact with their dam during specific periods of the day (Sirovnik et al. 2020). Further, there are systems with full cow-calf contact, meaning the cows and calves are managed together all day, except for temporarily separation for milking and feeding (Sirovnik et al. 2020). The different cow-calf contact systems comes with both advantages and disadvantages and this has been reviewed by Johnsen et al. (2016). A common concern regarding cow-calf contact systems is that there is an increased risk for impaired udder health in nursing dairy cows also milked by machine compared to cows solely milked by machine.

Intramammary inflammation, also known as mastitis, are one of the main causes for impaired udder health. Mastitis is usually caused by an intramammary infection (IMI) which in turn can be caused by several different pathogens (Zhao & Lacasse 2008). The most common pathogens causing IMI are bacteria such as *Staphylococcus* spp., *Streptococcus* spp., and *Escherichia coli* (Persson et al. 2011; Fernández et al. 2013). IMI is not only an economic and welfare problem; it also contributes to the use of antibiotics in the livestock industry (Carlén et al. 2004; Halasa et al. 2007; Bhutto et al. 2010). The risk of IMI is associated with parity, days in milk (DIM), teat end callosity (TEC), udder depth, cleanliness of the udder (Cardozo et al. 2015) and increased teat wall thickness (TWT) (Zecconi et al. 1996). Milking hygiene is also an important factor for the development of IMI.

Previous studies on udder health in nursing dairy cows have found varying results (Rasmussen & Larsen 1998; Fröberg et al. 2007; Barth 2020). During an experiment lasting for five weeks, Rasmussen and Larsen (1998) found that the teat skin condition was more impaired after suckling compared to the teats milked by machine. In the experiment the cow was suckled on one udder half while the other udder half was milked. When Barth (2020) studied full and part-time cow-calf contact systems, there were no significant difference in somatic cell count (SCC) between nursing cows and control cows. In a study of a part-time cow-calf contact system by Fröberg et al. (2007) the nursing cows had significant lower CMT scores compared to cows solely milked by machine. Further, the level of lactose was significantly lower in the control group, which could indicate impaired udder health. The suckling proceeded for 30-60 minutes after machine milking, which might have ensured the emptying of the udder.

de Passillé et al. (2008) found that there was a higher amount of residual milk in the cistern in nursing cows after they were milked by machine. Bruckmaier and Wellnitz (2008) have suggested that residual milk could be an excellent substrate for microorganisms, thus promote the risk for IMI. This was confirmed in a study by Penry et al. (2017a) who found a higher SCC as an effect of incomplete milking. However, Mendoza et al. (2010) could not find any signs of increased risk of IMI in part-time suckled cows. The calves drinking the residual milk could possibly be the explanation for the decreased risk of IMI, as the amount of substrate left for potential microorganisms is reduced. A higher oxytocin release during suckling compared to machine-milking has been reported (de Passillé et al. 2008), which may contribute to a better emptying of the udder when nursing.

In a full cow-calf contact system, there is a risk that calves suckle other cows than their dam, so called cross suckling. Fröberg and Lidfors (2009) studied the behavior of dairy calves in a voluntary milking system (VMS), and found that about 80% of the suckling bouts were on the dam. Das et al. (2000) also found that cross suckling occurred in a part-time cow-calf contact system. Calves suckling on various cows might be a potential way of spreading disease between cows in the herd.

Introducing calves into VMS could be advantageous compared to other milking systems, owing to automatic detachment of milking clusters at quarter level. Calves seem to prefer suckling of front teats (Fröberg & Lidfors 2009) which could entail uneven milk yield in different quarters. The automatic detachment at quarter level could decrease the risk of wearing of teats of quarters with lower milk yield. As mentioned earlier, both long-term effects like TEC, and short-term effects like increased TWT on the teat tissue caused by machine milking seem to be risk factors for mastitis (Zecconi et al. 1996; Cardozo et al. 2015). There is a common concern for dairy farmers that the combination of milking and suckling involves a high strain on the teats. However, the knowledge about the wearing of teats in cow-calf contact systems is limited. Therefore, this study primarily focuses on TEC and TWT as indicators for udder health.

The objective of this study is to examine the differences in TEC and TWT between nursing dairy cows also milked by machine (treatment) and a control group. The questions of issue are: 1) will there be a difference in TEC scores between treatment cows and control cows? 2) Does the teat wall respond differently to machine milking in treatment cows compared to control cows? The hypotheses are that there are no differences in TEC nor TWT between nursing dairy cows milked by VMS and cows solely milked by VMS.

2. Literature review

This literature review serves as a background for the collected data of TEC and TWT, as well as the results.

TEC and TWT are often mentioned together with SCC and milk flow. Therefore, a short introduction of these two parameters are presented first.

2.1. Somatic cell count

Somatic cells mainly consist of leukocytes (white blood cells) which reflects the level of inflammation in the mammary gland (Tsenkova et al. 2001; Schukken et al. 2003). The pathogens that cause mastitis can be divided into major and minor pathogens. Major pathogens are the ones that cause the greatest changes in milk composition, including increased SCC (Harmon 1994). Minor pathogens cause moderate inflammation, thus only a small rise in SCC (Harmon 1994). SCC are usually measured in the milk (Schukken et al. 2003), and is a widely used indicator for the cow's udder health (Tsenkova et al. 2001; Wall et al. 2018). In herd health recordings, SCC are commonly based on a combined sample of four quarters of two or more milkings (Schepers et al. 1997). A SCC greater than 200,000 cells/mL indicates subclinical mastitis (Tsenkova et al. 2001). SCC are also affected by physiological factors and are often elevated during the first days of lactation (Dohoo 1993; Barkema et al. 1999).

According to Olde Riekerink et al. (2007) SCC is influenced by age, stage of lactation, season, stress, management, day-to-day variation and diurnal variation. The risk of elevated SCC increases with larger teat canal diameter (Jorstad et al. 1989), parity and toward the end of lactation (Sheldrake et al. 1983; Schepers et al. 1997; de Haas et al. 2002; Guarín et al. 2017). Increased SCC has also been seen in cows with short milking intervals (Fernando & Spahr 1983; Hamann & Gyodi 2000).

The SCC can be measured at the cow-side by using a CMT. The CMT measures DNA of somatic cells in the milk (Jánosi & Baltay 2004; Plummer & Plummer 2012). A reagent is added to the milk DNA precipitate which leads to a change in viscosity of the reagent (Plummer & Plummer 2012). The degree of viscosity is scored subjectively as negative, +, ++ or +++ (Sargeant et al. 2001; Plummer &

Plummer 2012) and is directly related to the relative number of somatic cells in the sample (Plummer & Plummer 2012).

2.2. Milk flow

Milk flow is influenced by different factors. Sandrucci et al. (2007) found that the milk flow was affected by both parity and DIM, partly because of different milk yields. Tančin et al. (2006) found that milk flow was affected by DIM as well, while no relationship was found between milk flow and parity. Milk flow differs between quarters, both Weiss et al. (2004) and Tančin et al. (2006) found that peak flow rate and average flow rate was higher in rear quarters. Milk flow is also affected by anatomical traits such as teat canal length (TCL) and teat shape. Shorter teat canal and flat teat-end shape have been associated with a higher milk flow (Weiss et al. 2004; Wieland et al. 2017).

Grindal and Hillerton (1991) performed an experiment where cows with different milk flows was infected through a suspension of *Streptococcus agalactiae* and *S. dysgalactiae*. Cows with high flow rate quarters turned out to be more susceptible to infection. This is supported by Gäde et al. (2007) who found correlations between higher milk flow and increased SCC as well as increased susceptibility to mastitis. High peak milk flow rate have also been associated with milk leakage, which in turn is a risk factor for IMI due to enhanced bacterial growth in bedding material (Luttinen & Juga 1997; Klaas et al. 2005).

There seem to be a risk of impaired milk ejection in cow-calf systems when cows are milked by machine due to a reduced oxytocin release (Tancin et al. 2001; de Passillé et al. 2008), which in turn can result in reduced milk flow (Mendoza et al. 2010). One type of reduced milk flow emerge when the removal of cisternal milk occurs before the alveolar milk ejection (Sandrucci et al. 2007). This kind of insufficient milking have been associated with increased SCC (Sandrucci et al. 2005, 2007). According to Tančin and Bruckmaier (2015), a complete milk ejection at each milking is important for a high production level as well as good udder health. Today’s high producing dairy breeds has probably adapted to both hand- and machine milking. However, there are still some uncertainties around the oxytocin release during machine milking compared to suckling in nursing dairy cows (Tančin & Bruckmaier 2015). Tancin et al. (2001) suggests that the level of oxytocin release during milking also is influenced by the amount of milk in the udder. In their study they observed significantly lower oxytocin levels during milking preceded by two suckling bouts, compared to milking’s not proceeded by suckling.

The vacuum underneath the teat changes during milking (Besier & Bruckmaier 2016) and during high milk flow there is a decrease in vacuum at the teat tip (Ambord & Bruckmaier 2010). If the vacuum is too low there is a risk of liner slips and if it is too high there is a risk of damage on the teat tissue in the end of milking (Besier & Bruckmaier 2016). Thus, an accurate vacuum level is important to prevent lesions on the teat ends.

2.3. Teat end callosity

The primary barriers against pathogens entering the udder are the teat sphincter and the teat canal (de Pinho Manzi et al. 2012). It is therefore of high importance that those structures are in perfect shape to prevent IMI (de Pinho Manzi et al. 2012). Inside the teat canal the cells produce a layer of keratin which has antibacterial properties that prevent pathogens from entering the teat cistern (Zecconi et al. 2006). If the circulations of teat tissue fluids is impaired, by for instance mechanical forces from a milking machine, the function of the keratin layer as a barrier against pathogens might be inhibited (Hamann & Osteras 1994; Zecconi et al. 2006).

Mechanical forces from the milking machine causes the cells in the teat canal to produce excessive amount of keratin, so called hyperkeratosis (Hamann et al. 1994; Neijenhuis et al. 2000; Zoche-Golob et al. 2015) which disturbs the renewal of epithelial cells (Paulrud 2005). Callosity is the result from hyperkeratosis (Freeman 2002), thus the term teat end callosity (TEC) is used in this thesis. When the callosity ring is thick or rough the teat canal cannot close properly which facilitates the entry of microorganisms (Neijenhuis et al. 2001a).

The degree of TEC is associated with cow factors such as parity, milk yield, DIM, teat end shape, teat position, stage of lactation (Neijenhuis et al. 2000; Mein et al. 2001; Sandrucci et al. 2014; Pantoja et al. 2016), milk ability (Mein et al. 2001; Pantoja et al. 2016) and environmental factors such as duration of overmilking (Edwards et al. 2013). Overmilking is defined as when the milk flow through the teat canal is faster than the milk flow to the teat cistern (Rasmussen 2004).

According to Neijenhuis et al. (2001a) TEC increase in mid lactation compared to early and late lactation. Callosity formation was also studied by Shearn and Hillerton (1996), who found a rapid development of TEC in the first month of lactation which devolved in a gradual increase until about the fourth month of lactation. Later, in the end of lactation, TEC decreased. Both Neijenhuis et al. (2001a) and Shearn and Hillerton (1996) suggests that the variation over time could be explained by a higher milk yield, thus longer machine-on-time, during mid lactation.

An advantage of milking in VMS compared to conventional milking system (CMS) are the ability for VMS to milk individual quarters. Consequently, decreasing the risk of overmilking, which in turn is a risk factor for IMI (Natzke et al. 1982). Various studies have examined the effects on the teat tissue when changing from CMS to VMS (Berglund et al. 2002; De Vliegher et al. 2003). The studies found no significant differences in the score of TEC, although the milking frequency increased. Berglund et al. (2002) discusses that this might be a result of more gentle handling when milking at quarter level.

The results in studies of association between TEC and IMI varies. Guarín et al. (2017) found a correlation between SCC in quarters and scores as well as roughness for TEC at nine large milk producers in the US. This is in line with Cardozo et al. (2015), who also found that TEC was associated with SCC > 200,000 cells/mL. Breen et al. (2009b) studied risk factors for clinical mastitis, and found an increased risk for developing mastitis in cows with severe TEC. According to Zadoks et al. (2001) the rate of *Staph. aureus* infected quarters increased with extreme thickness of TEC, while quarters infected with corynebacteria increased in rough teat ends. However, TEC had no effect on the *Strep. uberis* infection rate (Zadoks et al. 2001). Melvin et al. (2019) found that teats with TEC had greater teat canal diameter after milking, suggesting with TEC thereby could be more susceptible to IMI. Gleeson et al. (2004) observed a significant correlation between TEC score and SCC in quarters not provided with disinfected. Yet, when post-milking disinfection was present there were no correlation. Zoche-Golob et al. (2015) could not find any associations between TEC and new IMI.

Breen et al. (2009a) presented a decreased risk for IMI (SCC > 200,000 cells/mL) in cows with mild or moderate TEC scores, suggesting a mild to moderate callosity ring might have a protective effect. This is supported by Pantoja et al. (2020) who concluded that mild TEC could be protective against subclinical mastitis, after summarizing research on the area. Paduch et al. (2012) found that higher TEC score was associated with the bacterial counts of *E. coli* and *Streptococcus uberis* in the teat canal. Yet, the bacterial count of *S. aureus* was not related to the degree of TEC. This indicates that the association between TEC and IMI could depend on which bacteria is present. Pantoja et al. (2020) reviewed several studies on the subject and concluded that severe TEC is a risk factor for both clinical and subclinical mastitis.

All previous studies on TEC have been executed in CMS. To our knowledge, there are no studies regarding the degree of TEC in nursing dairy cows that are also milked by VMS.

2.4. Teat wall thickness

The inner morphology of the teats and their alteration during milking is an important part of the defense against IMI and mastitis (Martin et al. 2018). The teat canal is designed to prevent milk from leaking out and to stop microorganisms from entering the udder (Paulrud 2005). When milking by machine short-term effects like an increase in teat length, TCL, TWT and teat end width (TEW) appear (Hamann & Mein 1990; Neijenhuis et al. 2001b; Szencziová et al. 2013; Strapák et al. 2018). The thickening of the teat is caused by congestion (intravascular accumulation of fluids) or oedema (extravascular accumulation of fluids) (Hamann et al. 1994; Paulrud 2005; Penry et al. 2017b). According to Hamann and Mein (1990), congestion is a precondition to oedema. Further, they discuss that machine-induced congestion should not have big influence on TWT post-milking because the blood vessels are designed to handle this change. Thus, the recovery from congestion of the teat tissue is fast. However, if oedema is developed, the blood supply to the teat tissue will decrease, and thus the time to recover will increase (Schulz 1971 see Hamann & Mein 1990).

The defense against IMI in the teat rely on an optimal blood supply to the teat tissue (Hamann & Osteras 1994; Zecconi et al. 2006). Further, the changes of the teat tissue during milking are assumed reflecting the penetrability of the teat canal (Neijenhuis et al. 2001b). Hamann et al. (1994) states that there is an increased risk of invasion of bacteria after milking due to the physiological changes in the teat.

There are several ways of measuring teat tissue reactions during milking. Some studies have used ruler (Guarín & Ruegg 2016) or 2-dimensional vision-based measuring technique (Zwertvaegher et al. 2013). Still, cutimeter (Hamann & Stanitzke 1990; Zecconi et al. 1996; Melvin et al. 2019), caliper (Hamann & Mein 1990; Stádník et al. 2010; Pařilová et al. 2011; Odorčić et al. 2020), and ultrasound (Neijenhuis et al. 2001b; Khol et al. 2006; Stádník et al. 2010; Pařilová et al. 2011; Szencziová et al. 2013; Martin et al. 2018; Melvin et al. 2019; Wieland et al. 2019; Odorčić et al. 2020) are the most common measuring techniques. All techniques except ultrasound measures external dimensions. Thus, when teat tissue reactions during milking are studied by ultrasound, internal dimensions like TWT, teat cistern and teat canal can be considered.

There is a spatial relationship between the TWT and the distension of the teat cistern. In-between milkings the intramammary pressure (IMP) increases the width of the teat cistern (Odorčić et al. 2020), which also can cause an expansion of the teat canal (Melvin et al. 2019). During milking the IMP decreases which leads to a decrease in teat cistern width (Neijenhuis et al. 2001b). Both Hamann and Mein (1990) and Odorčić et al. (2020) suggests that a reduction in IMP also affects the TWT change. When measuring the teat diameter by ultrasound the width of the teat cistern can be considered, thus the actual swelling of the teat wall can be determined. Neither Weiss et al. (2004) nor Melvin et al. (2019) could find any correlation between externally measured teat diameter and teat canal length. This indicates that the ultrasound method gives a more complete depiction of the teat.

Hamann et al. (1993) studied short-term reactions on the teat tissue when milking by machine at different vacuum levels (25 kPa; 30 kPa; 40 kPa; 50 kPa). The teat thickness, measured by caliper, at both the apex and barrel showed no alternation when milked at 25 and 30 kPa. Yet, they were significantly thicker after milking at 40 and 50 kPa, respectively. The teat apex as well as the teat barrel recovered within 30 minutes post-milking, with exception for teat barrel values when milked at 50 kPa, which was still significantly thicker 30 minutes post-milking.

Several studies have investigated the time it takes for the teat tissue to recover after milking (Neijenhuis et al. 2001b; Stádník et al. 2010; Szencziová et al. 2013; Melvin et al. 2019; Odorčić et al. 2020). In the study by Stádník et al. (2010) the TWT did not reach pre-milking values during the three hours duration of the trial. Szencziová et al. (2013) reported that the teat wall was significantly thicker 15 minutes post-milking compared to pre-milking values. At measurements 30, 45 and 60 minutes after milking the teat wall was still thicker than pre-milking, but not significantly so. However, the teat canal diameter reached pre-milking values within one hour. The data collection by Neijenhuis et al. (2001b) proceeded until eight hours after milking. The TWT reached pre-milking values within six hours. However, neither TEW nor TCL had recovered within eight hours. This is similar to the results by Melvin et al. (2019) where the TCL did not recover within the eight hour milking interval. Even though, the teat canal diameter did not change significantly during milking, there was an increase six to eight hours post-milking. The authors discuss that the IMP probably was the reason for the change. Odorčić et al. (2020) reported recovery of the TWT to pre-milking values within 35 minutes. The widely differing results could depend on the design and/or the milking technique used in the different trials. However, in the study by Odorčić et al. (2020), it took overmilked teats twice as long to recover. Thus, the occurrence of overmilking seem to affect the thickening and recovery of the teat wall. Pařilová et al. (2011) reported thicker teat wall and narrower teat cistern in overmilked teats, which was confirmed by Odorčić et al. (2020).

Thickening of the teat wall is not the only a reaction to milking. Several studies have reported a decrease in teat thickness after milking by machine (Hamann & Mein 1990; Zecconi et al. 1992, 1996). Hamann and Mein (1990) found a decrease at the teat apex, as measured by caliper, when milking by machine at 25 kPa and also when milking without pulsation at the teat apex. Zecconi et al. (1992) found a connection between a decrease in teat end thickness, as measured by cutimeter, and the usage of narrow-bore liners. Thus, a decrease in teat tissue thickness during milking can be explained by, for instance, equipment or machine settings (Hamann & Mein 1990; Zecconi et al. 1992).

Martin et al. (2018) used ultrasound measures to show that the teat wall and the teat length increased after milking, while the cistern and teat width decreased. On the contrary, Wieland et al. (2019) found that an increase in teat length after milking was associated with a decrease in TWT. Combined, these results indicate that the TWT depend on different physiological changes during milking and that a decreased teat width does not necessarily signify a decrease in TWT.

Hamann and Stanitzke (1990) compared the effects on the teat after suckling, hand milking and machine milking. They found that the teat end thickness decreased after suckling and hand milking while it increased after machine milking. The thickness of the teat end recovered within 15-30 minutes after suckling and hand milking, while it had not reached pre-milking values within 30 minutes after machine milking. In the same study, the increase in teat thickness was also bigger when milking by machine four times a day compared to two times a day.

Zecconi et al. (1992) studied the infection risk in dairy cows associated with teat tissue reactions to machine milking. They found that a change in teat thickness > 5% was associated to higher proportion of bacterial colonization. The risk of developing a new infection was also related to the change in thickness. Zecconi et al. (1996) found that quarters with an increase in teat thickness > 5% after milking was more likely to develop IMI. There was also a tendency that a decrease in teat thickness was associated with IMI. However, Zwertvaegher et al. (2013) found that a decrease in teat diameter at the barrel after milking were associated with lower SCC in quarters while an increase were associated with higher quarter SCC. Guarín and Ruegg (2016) could not find any association between anatomical change in teats post-milking and SCC. The inconsistent results between studies could depend on different ways of measuring teat thickness and different criteria for IMI.

It is clear that the changes of the teat wall during milking depends on different settings in the milking machine, such as vacuum level, pulsation rate and pulsation ratio (Hamann & Mein 1990; Zecconi et al. 1992, 1996; Hamann et al. 1993; Mein et al. 2001; Odorčić et al. 2020). All studies mentioned above, except Pařilová et al. (2011), were performed in CMS. Berglund et al. (2002) discovered that cows milked in a CMS had significantly thicker teat apex post-milking compared to pre-milking, while the change in cows milked by VMS was not significant.

To our knowledge, no previous studies have evaluated how TWT are influenced in nursing dairy cows milked by VMS.

3. Materials and Methods

This study is a part of an ongoing project at SLU, which is conducted at the Swedish Livestock Research Centre in Uppsala, Sweden. The project is approved by Uppsala Ethical Committee (ID: 5.8.18-18138/2019) and is divided into several batches. The current study include data from batch 1 and 2.

3.1. Housing and management

The aim of the overall project is to examine if cow calf contact can be integrated into VMS. Therefore, different ways to implement this have been tried out in the different batches. The common housing and routines for batch 1 and 2 are described first, while differences between the housing and management routines in the different batches are described below separate headlines.

Treatment cows and control cows, in both batches respectively, were housed in the same area within the Swedish Livestock Research Center, in a group of 50 cows in a VMS with controlled cow-traffic and cubicles. The cubicles were equipped with rubber mats, disinfectant (Stalosan®) and wood shavings. Wood shaving was automatically refilled four times a day and disinfectant two times a week. The cows had free access to roughage and water. The amount of concentrate was individually regulated and given through automatic dispensers as well as in the VMS-unit, a Delaval VMS™ Classic.

When calves were housed indoors, they stayed in a contact area to which the dams had access through a one- way selection gate. The contact area was equipped with cubicles, automatic concentrate dispensers for cows and water cups. Wood shavings in the contact area was automatically refilled seven times a day, while Stalosan® was refilled two times a week. In the contact area there was also a calf creep, where roughage, concentrate and water were provided to the calves. Cows could pass a one-way selection gate to get access to roughage, VMS-unit, and additional cubicles and concentrate dispensers. The design of the contact area was alike in both batches but was located in different parts of the VMS.

Standard settings in the VMS-unit were as follows: Vacuum level: 46kPa, Pulsation rate: 57-63 cycles/min, Pulsation ratio: 65:35, Milk flow at detachment: 0.5 kg/min and liners used was DeLaval Clover™.

After weaning some of the treatment cows and control cows were placed in new VMS within the Swedish Livestock Research Center due to readjustments in the herd. Consequently, some of the cows was thereby milked by a DeLaval VMS^{TM} V300. Regardless of VMS, all had the same milking settings and the resources was similar to the one described above.

The control cows followed the standard routines at the facility. The calving occurred indoors in a single pen and cows were moved to the VMS after the colostrum-period.

3.1.1. Batch 1

The first batch of cows and calves included 12 treatment cows and 12 control cows. The breeds were Swedish Holstein (n = 9) and Swedish Red and White (n = 15). Calves were born between 2019-08-14 and 2019-09-25. The mean parity number was 2.1 ± 1.2 and 1.8 ± 0.9 for treatment cows and control cows respectively.

The treatment cows calved outside in single pens in a mobile shelter and were divided into two groups. One group (n = 6) moved indoors into the VMS about 2-3 days post-partum. Although, they were allowed to go outdoors at night from 2019-09-04. The calves in the second group (n = 6) stayed outdoors on pasture until 2019-10-14. During the first day's post-partum, when cows and calves were kept in the calving pen, cows from both groups were fetched twice daily to be milked in the VMS-unit.

After the calving period, half of the mobile shelter was used to create an outdoor calf creep. The treatment cows used the other half of the shelter for resting. In the calf creep the calves had access to roughage, concentrate and water. The cows had access to water outside the contact area which they could leave through a selection gate. Moreover, the cows had continuous access to the VMS-unit, roughage and concentrate indoors in the VMS. When the calves were between 5-8 weeks old, they were moved indoors to the other cow-calf group together with their dams.

When treatment calves were on average eight weeks old, weaning was introduced by reduced contact time. The calves were confined in the calf creep during the night, but cows and calves could still see and smell each other. The full cow calf contact lasted for eight hours per day. The final weaning and separation were accomplished on the same day (2019-09-19). However, one of the calves were weaned 10 days prior the rest due to IMI in the dam. Thus, the age of the calves and DIM for the treatment cows at separation differed from 100 to 127 days. DIM for control cows at the same date varied between 85 and 108 days.

3.1.2. Batch 2

The second batch included 22 treatment cows and 19 control cows. The breeds were Swedish Holstein (n = 19) and Swedish Red and White (n = 22). Calves were born between 2020-03-03 and 2020-04-15. The mean parity number was 0.8 ± 1.1 and 1.5 ± 1.8 for treatment cows and control cows respectively. Cows who gave birth to heifers were prioritized to the treatment group due to long-term outcomes for the larger project. Calving occurred indoors in single pens and the cow-calf pairs was moved to the VMS 48-72 hours postpartum.

In the middle of May (2020-05-14) calves and contact area were moved to a pasture adjacent to the stable. The cows still had access to the stable with VMS-unit, roughage, concentrate and water. The calves could access a calf creep outdoors, provided with concentrate and water. Thus, calves had to leave the calf creep to suckle and they were not permitted to go into the barn. When the calves were between 12 and 18 weeks old (2020-07-06), they were provided with a nose flap that prevented suckling. The separation of the calves and dams was accomplished on the same day (2020-07-20), independent of birth date. Thus, the age of the calves and DIM for the treatment cows at weaning differed from 90 to 136 days. The DIM for control cows varied between 89 and 138 days for the same date.

3.2. Teat end callosity

Evaluation of the teats prepartum was performed in a loose house dry cow area with cubicles, while the evaluation at the later events was performed in the VMS-unit directly after detachment of milking clusters and before application of post milking teat disinfection. Milking permission was set to > 6 hours for the control cows and > 8 hours for treatment cows.

TEC was scored as smooth callosity ring and none (N), thin (1A), moderate (1B) or thick (1C), or rough callosity ring and thin (2A), moderate (2B), thick (2C) or extreme (2D) according to the teat end protocol developed by Neijenhuis et al. (2000). If the appearance of the teats were deemed in between two scores, the lower score was registered. At each observation any wounds on the teats were noted. During scoring, the observer wore plastic gloves that were changed between cows and used a flashlight and a mirror on a stick to be able to see the teat clearly.

3.2.1. Batch 1

Teats were scored on three occasions (Table 1), "postpartum"; about eight weeks after parturition (55 ± 12 DIM), "pre-weaning"; six days prior to weaning (107 ± 14 DIM), and "post-weaning"; eight weeks after weaning (163 ± 14 DIM). Three different observers conducted the scoring in the first batch.

3.2.2. Batch 2

The teat ends were scored at four occasions by the same observer (Table 1). “Prepartum”; 19 ± 10 days prior to calving, “postpartum”; eight weeks after calving (56 ± 3 days DIM), “pre-weaning”; three days prior to weaning (113 ± 25 DIM) and “post-weaning”; eight weeks after weaning (175 ± 18 DIM).

Table 1. Number of days pre- or postpartum when teat ends were scored, treatment and control cows merged.

	Batch 1	Batch 2
Prepartum		-19 ± 10
Postpartum	54 ± 9	56 ± 3
Pre-weaning	107 ± 14	113 ± 25
Post-weaning	163 ± 14	175 ± 18

3.3. Teat wall thickness

The TWT was assessed on 14 treatment cows and 13 control cows in the second batch. Treatment cows were 75 ± 11 DIM and control cows were 78 ± 12 DIM. Ultrasonographic pictures for estimating the TWT were taken via B-mode ultrasound sectioning with a 7.5 (4-9) MHz linear probe, set to 9 MHz, connected to a DRAMINSKI iScan. The procedure of scanning the teats was done according to Odorčić et al. (2020). The teats were submerged in the plastic cup, filled with lukewarm water. Contact gel was applied on the probe and the probe was held cranially on the plastic cup. The examination was performed by two trained people who altered between holding the probe and recording ultrasound pictures.

Cows included in the study were selected as they entered the VMS. The TWT was examined before and after milking for each teat. The before milking examination took place directly when the cow had entered the VMS, thus before udder preparation. After the pre-milking examination the VMS-unit was set into automatic mode and milking continued as usual. When all teat cups were attached the VMS-unit was again set to manual mode to allow for teat wall examination directly after detachment of milking cups (within two minutes from detachment). When all post-milking images were taken, the VMS-unit was set to automatic mode again and the teats were disinfected, and the cow let out.

When the memory of the ultrasound machine was full (~ 200 pictures), a first screening was performed, and the pictures were scored as having good or bad quality. The good quality pictures were used for further analysis. Subsequent, the pictures were erased, and the collection of additional pictures continued. The measurements of teat diameter (TD), teat cistern diameter (TCD) and TWT was performed on the ultrasound machine with 1-millimeter accuracy (Figure 1). TD, TCD and TWT measurements was taken 20 millimeters above the teat tip. The measurements were recorded in an excel file and the value for TWT was, in addition to the measurements, calculated as per [(TD-TCD)/2].

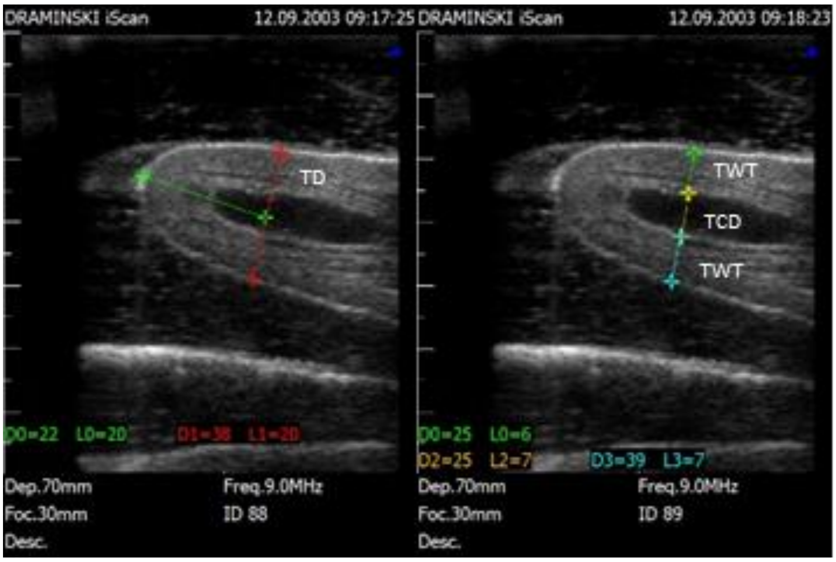

Figure 1. Pictures demonstrating where the measurements for teat diameter (TD), teat cistern diameter (TCD) and teat wall thickness (TWT) were taken.

3.4. Statistical analysis

3.4.1. Teat end callosity

Before the statistical analysis was performed, the TEC scoring system (N; 1A; 1B; 1C; 2A; 2B; 2C; 2D) was modified to a scoring system based on numbers to calculate the mean scores and simplify the data analysis (Table 2). N was scored as 0, 1A was scored as 1, 1B was scored as 2 and so on. The mean and standard deviation (SD) of the scores for treatment cows and control cows, in each batch respectively and together, were calculated both by excel and Paleontological Statistics, version 4.05 (PAST). The values were then inserted in diagrams (figure 2; figure 3; figure 4). Data were checked for normal distribution by using Anderson-Darling normality test in PAST. It was found out that none of the data were normally distributed. Thus, to determine any differences in means between treatment cows and control cows, data were analyzed with the Mann-Whitney U-test. First, data from batch 1 and batch 2, were analyzed separately. Secondly the two batches were compiled and analyzed together. The null hypothesis (H_0) was that the two groups had equal means. Further, the transformation of the TEC score during lactation within each group was analyzed for significant changes in a Mann-Whitney U-test.

Table 2. Conversion of scores.

Score	Data
N	0
1A	1
1B	2
1C	3
2A	4
2B	5
2C	6
2D	7

As for calculating the statistical power the program G*Power (Version 3.1.9.8; Faul et al. 2007) was used. Test family was set as t test, statistical test was "Means: Wilcoxon-Mann-Whitney test (two groups)", and the type of power analysis was "Post hoc: Compute achieved power – given α, sample size, and effect size". In the post hoc analysis the power (1 - β) is computed as a function of α, the population effect size parameter, and the sample sizes in the study. Means and SD for treatment cows and control cows from each scoring event was inserted as parameters of which the effect size was calculated.

3.4.2. Teat wall thickness

The data for TWT before and after milking were statistically analyzed in PAST. The calculated mean difference (in mm and as % change) between measurements before and after milking for treatment cows and control cows respectively were put into boxplots to demonstrate the median, minimal, and maximal values in TWT change. The data were analyzed for normality in PAST using an Anderson-Darling normality test. The data were normally distributed, thus a two-sample t-test was performed to evaluate if TWT changed in the respective groups. The H_0 was that the two groups (treatment; control) had equal means. Further, another two-sample t-test with the same H_0 was performed to discover any differences between the two groups (treatment; control). Secondly, time since last milking was considered and included in the analysis. Time was categorized into three intervals: < 10 hours since last milking, 10-20 h since last milking and > 20 hours since last milking. When time was considered the three groups were compared to each other. Therefore, Mann-Whitney pairwise test with Bonferroni corrected p-values was used.

To test the statistical power the program G*Power was used. For test family, t test was used, and for statistical test the "Means: difference between two independent means (two groups)" was used. The type of power analysis that was used was "Post-hoc: compute power achieved power – given α, sample size, and effect size". To determine the effect of the sample size the means for TWT change (mm) and SD was inserted in the model.

4. Results

4.1. Teat end callosity

4.1.1. Batch 1

For the analysis of the results in batch 1 three cows from each group were excluded due to missing data or differences in the management routines. Thus, the total amount of scored cows included in the results were 19 (treatment = 9; control = 10). This resulted in 108 teat assessments in treatment cows and 120 teat assessments in control cows, a total of 228 assessments (Table 3).

Table 3. Number of cows, cows in each parity and number of assessments in the different batches.

		Batch 1		Batch 2		Total	
		Treatment	Control	Treatment	Control	Treatment	Control
Cows		9	10	21	16	30	26
	Parity 1	5	5	12	6	18	11
	Parity 2	3	4	4	5	7	9
	Parity 3	1	1	4	2	5	3
	Parity 4	0	0	1	2	2	1
	Parity 5	0	0	0	1	0	1
Prepartum		0	0	83	64	83	64
Postpartum		36	40	83	64	119	104
Pre-weaning		36	40	83	64	119	104
Post-weaning		36	40	79	64	115	104
Total		108	120	328	256	436	376

Only at five assessments were teats scored as rough (treatment = 0; control = 5), while the rest was scored as smooth (Table 4). Postpartum the mean score ± SD for treatment cows was 0.5 ± 0.65 while for control cows the mean score was 1.2 ± 1.3 (Table 5). Prior to weaning the mean score was 1.39 ± 0.55 and 1.2 ± 1.04 for treatment cows and control cows respectively. At the last scoring event, post-weaning, the mean score for both treatment cows and control cows was 1.28, with a SD of 0.61 for treatment cows and 0.86 for control cows.

The transformation of mean scores are described in Figure 2. The change in treatment cows from postpartum to pre-weaning was significant ($p < 0.001$), while the change from pre-weaning to post-weaning was not. Likewise, the change from postpartum to post-weaning was significant ($p < 0.001$). The scores for control cows did not change significantly during the study.

Table 4. Distribution of teat end callosity (TEC) scores and wounds in numbers (n) and % in treatment cows and control cows in batch 1.

	Postpartum				Pre-weaning				Post-weaning			
	Treatment		Control		Treatment		Control		Treatment		Control	
Score	n	%	n	%	n	%	n	%	n	%	n	%
N	21	58.3	15	37.5	1	2.8	11	27.5	3	8.3	6	15
1A	12	36.4	13	32.5	20	55.6	14	35	20	55.6	21	52.5
1B	3	8.3	5	12.5	15	41.7	13	32.5	13	41.7	9	22.5
1C			3	7.5			1	2.5			4	10
2A			4	10								
2B							1	2.5				
2C												
Wound	3	8.3	1	2.5	1	2.8	1	2.5				

Postpartum: 54 ± 9 days in milk (DIM), pre-weaning: 107 ± 14 DIM and post-weaning: 163 ± 14 DIM.

Table 5. Mean teat end callosity (TEC) score ± standard deviation in batch 1 at the different scoring events in treatment cows and control cows respectively. Table also displays days in milk (DIM) at each scoring event.

Scoring event (DIM)	Treatment	Control
Postpartum (54 ± 9)	0.50 ± 0.65^{a}	1.20 ± 1.30^{b}
Pre-weaning (107 ± 14)	1.39 ± 0.55^{b}	1.20 ± 1.04^{b}
Post-weaning (163 ± 14)	1.28 ± 0.61^{b}	1.28 ± 0.85^{b}

Different letters mean significant difference ($^{a} \neq {}^{b}$).

When the results were analyzed for statistical power the power was 88.7% postpartum, 24.5% pre-weaning and 5.2% post-weaning. This means that the significant difference between the treatment cows and control cows postpartum was not due to chance with 88.7% power. The number of teats pre-weaning was enough to identify a difference of 0.50 with 80% power and 95% confidence interval. As for post-weaning the number of teats was enough to identify a difference of 0.44 at 80% power and 95% confidence interval.

Wounds on teats

Regarding wounds on the teats, six teats (treatment = 4; control = 2) displayed wounds at any point during the study. All wounds observed were superficial and only affecting the outer most skin layer. All wounds observed were healed before next scoring event. Four of the wounds were noticed at the postpartum TEC scoring (treatment = 2; control = 1). One of the treatment cows was injured on two teats, left front: 3x3 mm and left rear: 1x10 mm. Another treatment cow was injured on the left rear teat with a size of 1x2 mm was observed. At the same scoring event a control cow had a wart with a scab on the right rear teat that was classified as a wound. At the pre-weaning scoring event, the same control cow had a wound on her right front teat and one treatment cow had an old scab at her left rear teat.

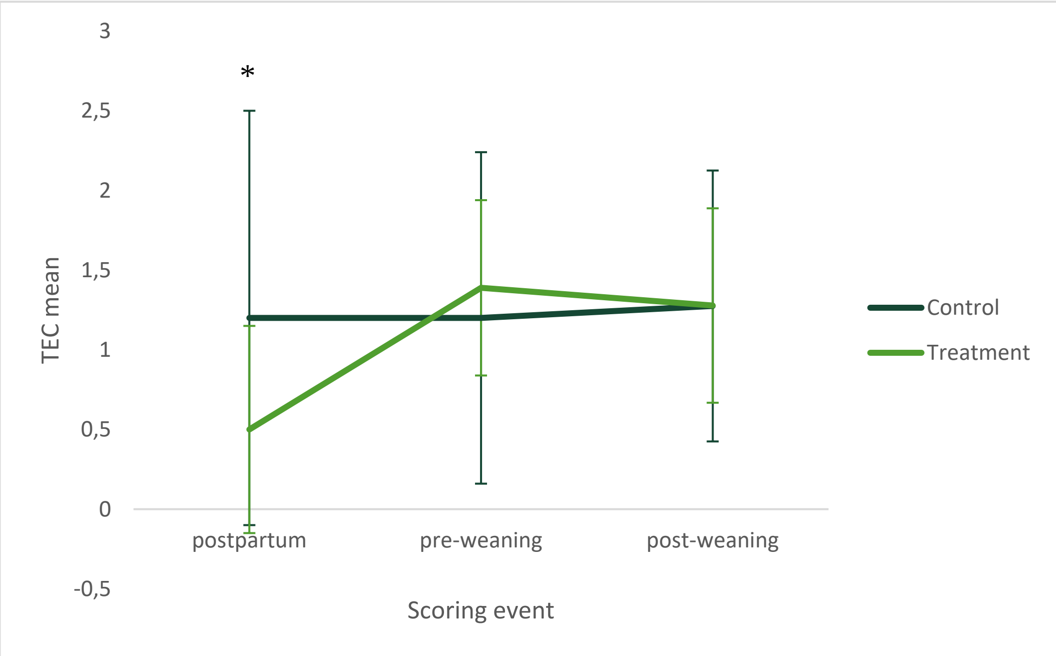

Figure 2. Transformation of mean teat end callosity (TEC) scores during lactation for treatment cows and control cows in batch 1. Whiskers displays the standard deviation (= significant (p < 0.05))*

4.1.2. Batch 2

In the second batch two control cows was excluded due to injuries and one was culled due to mastitis. One of the treatment cows was moved to a sick pen about four weeks postpartum due to an injury on her left front teat. The injured teat was dried off and excluded from this project. During the approximately three weeks in sick pen, the three healthy teats were suckled by the calf and machine-milked in a bucket. Another treatment cow was culled due to mastitis about a month after weaning and was thereby excluded from the last scoring event (post-weaning). The total amount of scored teats was 143 (treatment = 79; control = 64), and the four scoring events resulted in 572 assessments (Table 3). The scores were distributed according to Table 6.

Before calving most teats were scored as N while the variation increased during lactation, demonstrated in Figure 4. The transformation of the TEC was significant from prepartum to postpartum ($p < 0.001$), for both treatment cows and control cows. Yet, for none of the groups, neither the transformation from postpartum to pre-weaning nor from pre-weaning to post-weaning was significant. However, both the transformation from prepartum to pre-weaning and post-weaning, respectively, was significant ($p < 0.001$) in both groups.

Table 6. Distribution of teat end callosity (TEC) scores and wounds in numbers (n) and % in treatment cows and control cows in batch 2.

	Prepartum				Postpartum				Pre-weaning				Post-weaning			
	Treatment		Control		Treatment		Control		Treatment		Control		Treatment		Control	
Score	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
N	82	98.8	60	93.8	20	24.1	15	23.4	23	27.7	8	12.5	13	16.5	9	14.1
1A	1	1.2	4	6.3	33	39.8	29	45.3	37	44.6	34	53.1	44	55.7	35	54.7
1B					27	32.5	16	25	22	26.5	19	29.7	15	19.0	12	18.8
1C					2	2.4	4	6.3	1	1.2	3	4.7	7	8.9	8	12.5
2A					1	1.2										
2B																
2C																
Wound					2	2.4			1	1.2						

Days prepartum: 19 ± 10. Postpartum: 56 ± 3 DIM, pre-weaning: 113 ± 25 DIM and post-weaning: 175 ± 18 DIM.

There was only one teat that was scored as rough at one assessment, while the others were scored as smooth. Before calving the mean score ± SD was 0.01 ± 0.11 and 0.06 ± 0.24 for treatment cows and control cows, respectively (Table 7). Postpartum the mean score was 1.17 ± 0.87 and 1.14 ± 0.85 for treatment cows and control cows, respectively. At the third scoring event (pre-weaning) the mean score for treatment cows was 1.01 ± 0.77 and for control cows the mean score was 1.27 ± 0.74. After weaning the mean score was 1.20 ± 0.82 and 1.30 ± 0.87 for treatment cows and control cows respectively.

Table 7. Mean teat end callosity (TEC) score ± standard deviation in batch 2 at the different scoring events in treatment cows and control cows respectively. Table also displays days prepartum and days in milk (DIM) at each scoring event.

Scoring event (DIM)	Treatment	Control
Prepartum (-19 ± 10)	0.01 ± 0.11[a]	0.06 ± 0.24[a]
Postpartum (56 ± 3)	1.17 ± 0.87[b]	1.14 ± 0.85[b]
Pre-weaning (113 ± 25)	1.01 ± 0.77[b]	1.27 ± 0.74[b]
Post-weaning (175 ± 18)	1.20 ± 0.82[b]	1.30 ± 0.87[b]

Different letters mean significant difference ([a] ≠ [b]).

When the results were tested for statistical power the result was 47.2% prepartum, 7.3% postpartum, 62.5% pre-weaning and 16% post-weaning. The number of assessed teats prepartum was enough to identify a difference of 0.08 at 80% power and 95% confidence interval. Postpartum the number of teats was enough to find a difference of 0.37 with 80% power and 95% confidence interval. The number of teats pre-weaning was enough to identify a difference of 0.33 at 80% power and 95% confidence interval and at last, the number of teats assessed post-weaning was enough to find a difference of 0.37 with 80% power and 95% confidence interval.

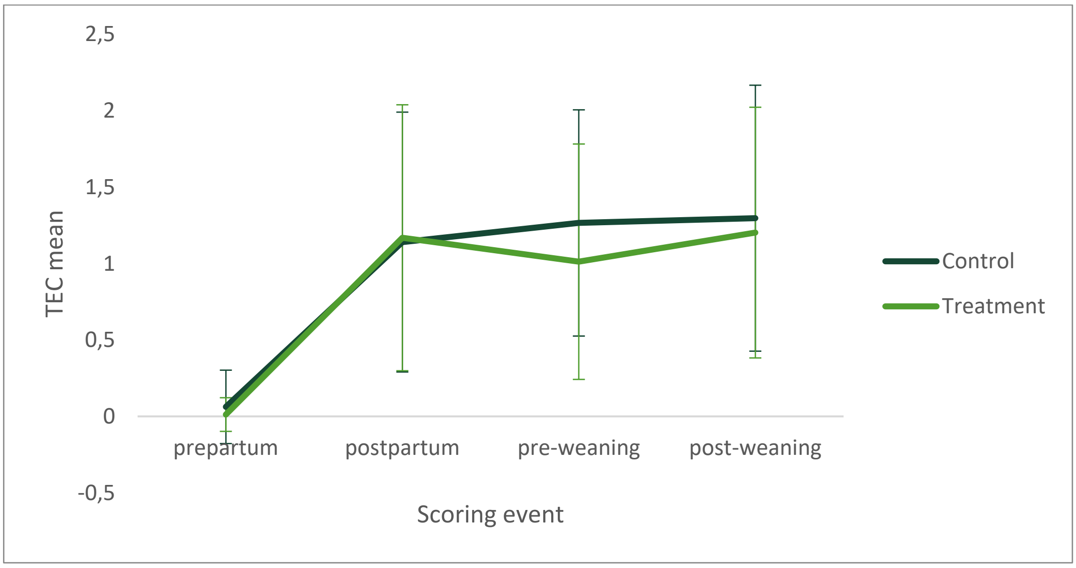

Figure 3. Transformation of mean teat end callosity (TEC) scores during lactation for treatment cows and control cows in batch 2. Whiskers displays the standard deviation. The transformation in treatment cows and control cows from prepartum to postpartum is significant (p < 0.001).

Wounds on teats

No wounds were noticed at the TEC scoring before calving nor after weaning. At the scoring event postpartum one of the treatment cows had wounds on both front teats. On the right front teat, the wound was about 1x7mm placed halfway up from the teat apex, craniolaterally. On the left front teat, there was a scab which had the same location as the wound on right front teat but was about 5 mm long. At the scoring event prior to weaning the wounds found during the previous scoring event were healed. At the scoring event pre-weaning a wound on left front teat on one of the treatment cows was found. The wound was vertically, had a size of about 4x20 mm and had a cranial location. This wound too had healed before the next scoring event. All wounds observed were superficial and only affecting the outer most skin layer.

4.1.3. Batch 1 & 2

When batch 1 and 2 were merged, the treatment group consisted of 30 cows and the control group included 26 cows (Table 3). The total number of assessments were 812 (treatment = 436; control = 376). There were no significant differences ($p > 0.05$) between treatment cows and control cows at any of the scoring events. The scores were distributed as per Table 8.

Table 8. Distribution of teat end callosity (TEC) scores and wounds in numbers (n) and % in treatment cows and control cows in batch 1 and 2 merged.

	Prepartum				Postpartum				Pre-weaning				Post-weaning			
	Treatment		Control		Treatment		Control		Treatment		Control		Treatment		Control	
Score	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
N	82	98.8	60	93.8	41	34.4	30	28.8	24	20.2	19	18.3	16	13.9	15	14.4
1A	1	1.2	4	6.3	45	37.8	42	40.4	57	47.9	48	46.2	64	55.7	56	53.8
1B					30	25.2	21	20.2	37	31.1	32	30.8	28	24.3	21	20.2
1C					2	1.7	7	6.7	1	0.8	4	3.8	7	6.1	12	11.5
2A					1	0.8	4	3.8								
2B											1	0.9				
2C																
Wound					5	4.2	1	0.9	2	1.7	1	0.9				

Days prepartum = 19 ± 10. Postpartum: 54 ± 9 DIM, pre-weaning: 113 ± 25 DIM and post-weaning: 171 ± 22 DIM.

The data for the scoring event prepartum is from batch 2 alone while the other scoring events includes data from both batches. The mean score prepartum was 0.01 ± 0.11 and 0.06 ± 0.24 for treatment cows and control cows respectively (Table 9). Postpartum the mean score was 0.97 ± 0.86 and 1.16 ± 1.04 for treatment cows and control cows, respectively. Prior to weaning the score for treatment cows was 1.13 ± 0.73 and for control cows the mean score was 1.24 ± 0.86. At the last scoring event, post-weaning, the mean scores were 1.23 ± 0.76 and 1.29 ± 0.86 for treatment cows and control cows, respectively.

Table 9. Mean teat end callosity (TEC) scores ± standard deviation in treatment cows and control cows at the different scoring events when batch 1 and 2 were emerged. Table also displays days prepartum and days in milk (DIM) at each scoring event.

Scoring event (DIM)	Treatment	Control
Prepartum (-19 ± 10)	0.012 ± 0.110[a]	0.063 ± 0.244[a]
Postpartum (54 ± 9)	0.966 ± 0.863[b]	1.164 ± 1.044[b]
Pre-weaning (113 ± 25)	1.126 ± 0.731[b]	1.240 ± 0.865[b]
Post-weaning (171 ± 22)	1.226 ± 0.762[b]	1.289 ± 0.855[b]

Different letters mean significant difference ([a] ≠ [b]).

The transmission of TEC scores is demonstrated by Figure 4. The change from prepartum to postpartum was significant ($p < 0.001$) for both treatment cows and control cows. However, the further transmission as the lactation proceeded was not significant in neither treatment cows nor control cows.

When the results were tested for statistical power the result was 47.2% for prepartum, 44.2% postpartum, 27% pre-weaning and 14% post-weaning. The number of teats prepartum was enough to find a difference of 0.08 with 80% power and 95% confidence interval. Postpartum the number of teats was enough to find a difference of 0.33 with 80% power and 95% confidence interval. Further, pre-weaning the number of teats was enough to find a difference of 0.28 with 80% power and 95% confidence interval. As for post-weaning the number of teats was enough to find a difference of 0.28 at 80% power and 95% confidence interval.

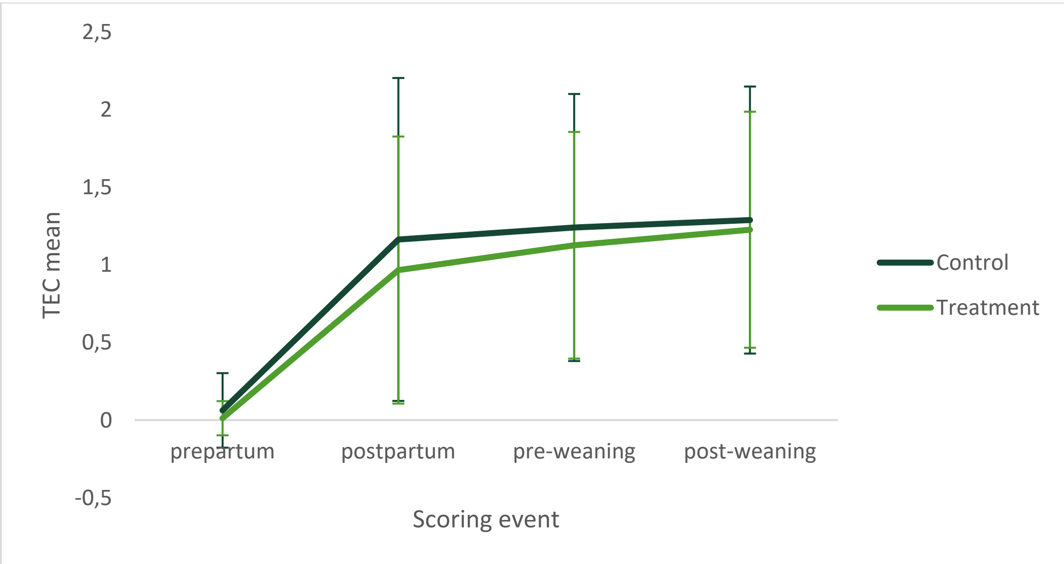

Figure 4. Transmission of mean teat end callosity (TEC) scores in batch 1 and 2 merged, during lactation. Whiskers displays the standard deviation.

4.2. Teat wall thickness

A total of 269 images was taken, of which 180 images was scored as good and had matching pre- and post-milking images from the same teat. The analyzed images were from 14 treatment cows and 13 control cows (Table 10). The total amount of teats for the analysis was 46 and 44 from treatment cows and control cows respectively. Every teat was measured before and after milking, thus, the number of measurements was 92 for treatment cows and 88 for control cows.

All teat walls except one increased during milking. The teat that decreased in TWT during milking was appertained to a second parity control cow which was milked about seven hours before the measurements, and the decrease was 0.5 mm. The mean ± SD thickness of teats belonging to treatment cows was 5.641 ± 1.260 mm and 7.955 ± 1.090 mm before and after milking respectively (Table 11; figure 5). Thus, the thickness of the teats in the treatment group increased on average 2.315 ± 1.384 mm, or 46.68% ± 33.45% after milking ($p < 0.001$), demonstrated by Figure 7 and 8, respectively. The mean TWT in control cows was 5.307 ± 1.207 mm and 8.148 ± 1.283 mm before and after milking respectively (Table 11; figure 6). Thus, the thickness of the control cows' teats increased by 2.849 ± 1.572 mm or 58.85% ± 41.17% on average after milking ($p < 0.001$), demonstrated by figure 7 and 8, respectively. There was no difference between the groups in how much the TWT increased during milking.

Table 10. Number of cows, teats and measurements of teat wall thickness (TWT) in treatment cows and control cows respectively.

		Treatment	Control
Cows		14	13
	Parity 1	7	6
	Parity 2	1	4
	Parity 3	4	3
	Parity 4	2	0
Teats		46	44
	Front teats	25	22
	Rear teats	21	22
Total number of measurements		92	88
Measurements pre-milking		46	44
Measurements post-milking		46	44

When time since last machine milking was taken into consideration (Figure 9; 10), there were only control cows (n = 6) in the interval < 10 hours since last milking. Furthermore, for the interval > 20 hours since last milking there were only treatment cows (n = 10). As for the interval 10-20 hours since last milking there were both treatment cows (n = 5) and control cows (n = 8). However, there were no significant difference in TWT change between the three intervals (p > 0.05).

The power of the statistical analysis of change (mm) in TWT post-milking in treatment cows compared to control cows was calculated to 55%. The number of measurements in the present study would have been enough to find a difference in TWT of 0.75 mm with 80% power and 95% confidence interval.

Table 11. Means and standard deviation for teat wall thickness (TWT) pre- and post-milking, as well as the change in mm and %.

	Treatment	Control	P-value
TWT pre-milking (mm)	5.641 ± 1.260[a]	5.307 ± 1.207[a]	0.202
TWT post-milking (mm)	7.955 ± 1.090[b]	8.148 ± 1.283[b]	0.449
Change (mm)	2.315 ± 1.384	2.849 ± 1.572	0.094
Change (%)	46.677 ± 33.450	58.850 ± 41.173	0.128

Different letters indicate significant difference ([a] ≠ [b]).

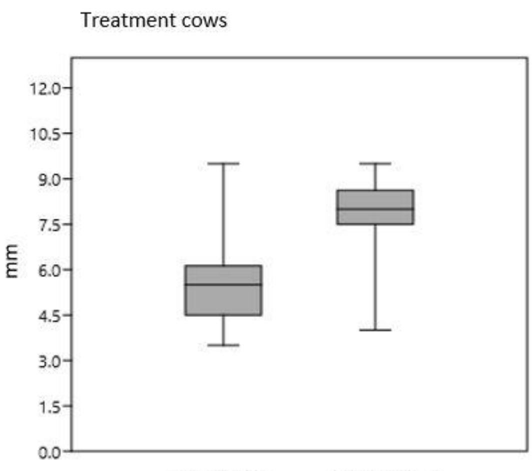

Figure 5. Boxplot of pre- and post-milking values in treatment cows. Horizontal line inside the box displays the median and whiskers displays the maximum and minimum value.

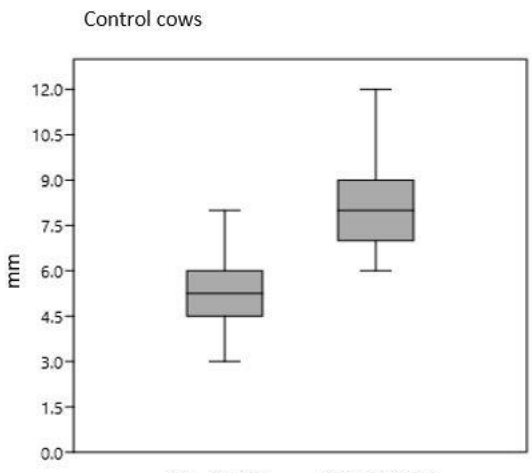

Figure 6. Boxplot of pre- and post-milking values in control cows. Horizontal line inside the box displays the median and whiskers displays the maximum and minimum value.

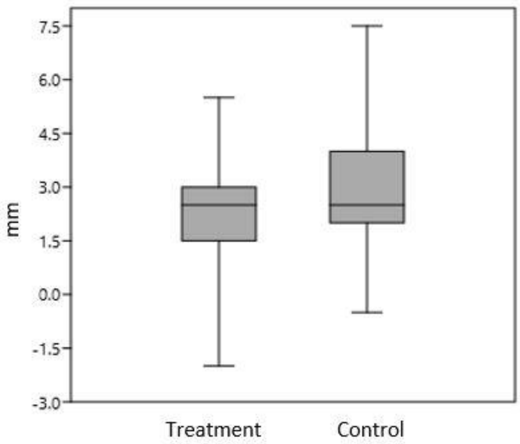

Figure 7. Teat wall thickness (TWT) change during milking in mm for treatment cows and control cows. Horizontal line inside the box displays the median and whiskers displays the maximum and minimum value.

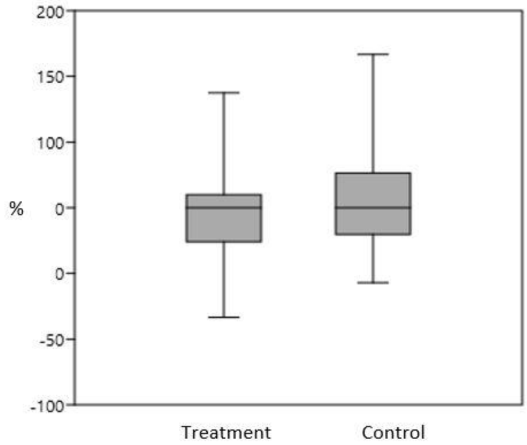

Figure 8. Teat wall thickness (TWT) change in % during milking for treatment cows and control cows. Horizontal line inside the box displays the median and whiskers displays the maximum and minimum value.

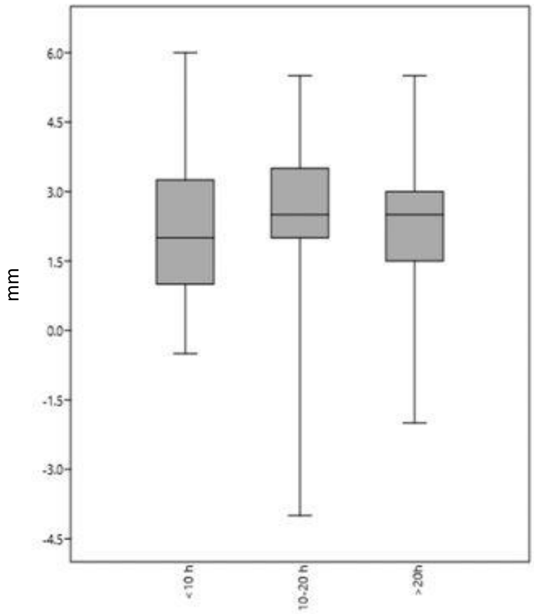

Figure 9. Boxplot demonstrating change of teat wall thickness during milking in mm, relative to time since last milking event. Horizontal line inside the box displays the median and whiskers displays the maximum and minimum value.

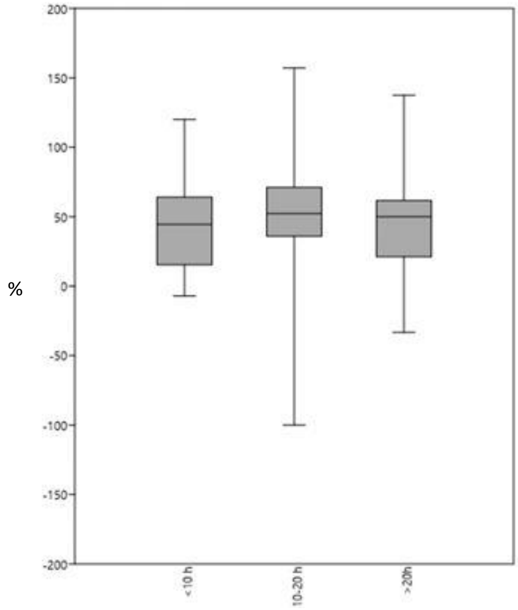

Figure 10. Boxplot demonstrating change of teat wall thickness during milking in %, relative to time since last milking event. Horizontal line inside the box displays the median and whiskers displays the maximum and minimum value.

5. Discussion

The aim of this MSc thesis was to investigate if short-term (TWT) and long-term (TEC) effects on the teat tissue caused by milking differ between nursing dairy cows (also milked by VMS) and cows solely milked by machine. The primary research questions were: 1) will there be a difference in TEC scores between treatment cows and control cows? 2) Does the teat wall respond differently to machine milking in treatment cows compared to control cows?

5.1. Teat end callosity

Treatment cows did not have higher TEC scores compared to control cows at any of the scoring events. There was a significantly lower degree of TEC in treatment cows at one occasion, namely postpartum in batch 1, with a statistical power of 88.7%. At all other scoring events or when batch 1 and 2 were merged, no significant differences between treatment cows and control cows in mean TEC scores were found.

In batch 2 TEC mean scores increased significantly in both groups from prepartum to postpartum. To our knowledge, TEC scoring prepartum have not been conducted before. The results in the present study showed that 142 teats of 147 had no callosity ring at all prepartum. The other five was scored as 1A. This indicates that TEC mostly recover during dry period, but not always. One of the cows scored with 1A was a first parity cow. A possible reason could be that she has been cross-suckled. According Neijenhuis et al. (2000), Espe and Cannon (1942) and Sieber and Fransworth (1984) states that hyperkeratosis can arise in suckler cows and hand milked cows as well.

From eight weeks postpartum there was a very slight, non-significant increase in TEC mean scores throughout the lactation in control cows in both batches. However, the transformation of TEC in treatment cows during lactation differed between batch 1 and 2. TEC mean scores for treatment cows in batch 2 decreased pre-weaning (~ 4 months postpartum) and increased post-weaning (~ 5 months postpartum) (not significant). This is in contrast to Shearn and Hillerton (1996) who reported an increase in average TEC score until about 4 months postpartum and a decrease from about 6-7 months postpartum. The increase in TEC mean scores post-weaning in batch 2 could possibly be attributed to the weaning, thus a higher milk yield in the machine and longer machine-on-time. In batch 1 the increase from postpartum to pre-weaning for treatment cows was significant. The transformation was similar to the one reported by Shearn and Hillerton (1996).

The disparity in score change in batch 1 and 2 could also depend on the execution of scoring. In batch 1 the scoring was performed by three different observers, while the scoring in batch 2 was performed by the same observer. Even though all three were trained by the same person, this could be an explanation of the different results.

In the present study, treatment cows had slightly lower TEC mean scores compared to control cows when batch 1 and 2 were emerged (not significant). As mentioned in the literature review the degree of TEC is influenced by milk yield and DIM (Neijenhuis et al. 2000; Mein et al. 2001), thus the machine-on-time. The adjustments of milking interval in the VMS-unit was set to eight hours for treatment cows and six hours for control cows. Thus, the treatment cows could potentially be milked by machine three times a day, while the control cows could be milked four times a day. Since the treatment cows also were suckled the milk yield to the machine would be lower compared to control cows. Consequently, they would experience shorter machine-on-time and hence less wearing of the teats.

Both Shearn and Hillerton (1996) and Neijenhuis et al. (2000) reported a larger proportion of more severe callosity rings than the present study. The low amount of rough callosity rings in this study might be assigned to the milking technique and milking machine settings. Few studies included in this thesis specify the level for detachment of milking clusters. Notably, all were made in CMS which devote detachment of milking clusters at udder level. Edwards et al. (2013) used a detach level of 0.2 kg/min for the control group when studying overmilking in CMS. In the present study, the detachment at quarter level was set to 0.5 kg/min. A higher detach level decreases the risk for overmilking, thus the risk for a higher degree of TEC (Edwards et al. 2013). Other parameters like teat shape also affects the TEC. Neijenhuis et al. (2000) reported more severe TEC in pointed and round teats compared to inverted teats. However, the present study did not include evaluation of the teat shape, therefore the lower degree of TEC cannot be assigned to this.

As for the method of scoring teats, there was some difficulties to interpret the pictures at the scoring scheme. Many of the teats were scored as 1A or 1B, which, on the scheme, was very similar. When the decision was hard to make, the routine was to choose the minor callosity ring. At the same time, the pictures representing higher scores was not hard to interpret. Since the higher TEC scores seem to be more connected to the risk for mastitis (Zadoks et al. 2001; Breen et al. 2009a), it is of higher importance to be able to distinguish between those. Also, most studies on TEC are made in CMS which might entail a higher degree of TEC. The interpretation of the scoring scheme might be easier when TEC are more distributed between higher scores because of the greater difference between the pictures. Therefore, it might be an advantage to develop another scoring scheme adapted to teats milked by VMS. Such scoring scheme should focus on the lower scores of TEC and display more degrees of callosity corresponding N, 1A, 1B and 1C.

When converting the TEC scores to numbers for the data analysis the difference between the scores were not considered. As mentioned earlier, the lower TEC scores were more difficult to distinguish between, while there was a bigger difference between the more severe TEC scores. Consequently, the conversion of scores would have been more accurate if the scale was fitted to the difference between the TEC scores, and not a gradually increasing scale.

Wounds were found on the teats at 9 of the 812 TEC assessments (Batch 1 = 6; Batch 2 = 3). Six of the wounds (treatment = 5; control = 1) were detected postpartum, thus about eight weeks into lactation. The other three (treatment = 2; control = 1) were found pre-weaning. Almost 78% of the wounds were found on teats belonging to the treatment cows. However, as the number of wounds was scarce, it is hard to identify the exact cause. All wounds on the teats were also healed at the succeeding scoring event which indicates that they did not originate from suckling.

Whether the relatively low TEC scores in both treatment cows and control cows would increase the risk for IMI and mastitis is unclear. A comparison between TEC and SCC, for instance, could have given an indication of the udder health. However, as previous studies have presented (Zadoks et al. 2001; Breen et al. 2009a), the highest risk for IMI seem to be associated to thick or rough callosity rings, which was rarely seen in this study.

As for future research, the degree of TEC should be evaluated in different types of cow-calf contact systems. How TEC is affected by part-time suckling would be an interesting question. Part-time suckling might influence the milk yield in the machine, thereby the machine-on-time. Previous studies have also reported a higher bacterial load on teats with TEC (Zadoks et al. 2001; Paduch et al. 2012). If pathogens are spread between nursing cows through suckling, what are the most common pathogens? And are they more common to find on teats exposed to TEC? Those are questions significant to answer.

5.2. Teat wall thickness

There were no significant differences in TWT change during milking between the treatment cows and control cows, thus the teat wall does not seem to respond differently to machine-milking in treatment cows compared to control cows. However, the increase in TWT was slightly less in treatment cows compared to control cows (not significant). As mentioned previously, the milking interval between the two groups differed when the TWT was assessed. A larger proportion of treatment cows was milked by machine with an interval of > 20 hours while a larger proportion of control cows was milked by machine with an interval of < 10 hours. During the 2 weeks prior to the data collection the cows were not allowed to enter the stable between 8 and 12 am due to reconstruction in the facility. During this period the milking intervals in the VMS-unit was disturbed and the number of milkings per day probably decreased in comparison with regular milking intervals in both groups. Yet, treatment cows were still suckled by calves. The suckling probably entailed lower milk yield in the VMS-unit, thus shorter machine-on-time, for treatment cows. This might be an explanation for the smaller increase in TWT during milking compared to control cows. Previous studies have reported thicker teat walls after overmilking which indicates that machine-on-time might have an effect on the teat tissue (Pařilová et al. 2011; Odorčić et al. 2020).

Further, we investigated if the milking interval could affect the reaction of the teat tissue, but no significant results were found. However, when the treatment cows last was suckled is unknown, thus the data for milking intervals is uncertain. The longer interval between milkings by machine could have allowed for complete recovery of the teat tissue before next milking. Studies have displayed changes in TEW and TCL during milking which did not recover within eight hours (Neijenhuis et al. 2001b; Melvin et al. 2019). However, Neijenhuis et al. (2001b) reported that the TWT recovered within six hours post-milking. The recovery before next milking is advantageous since it decreases the risk for invasion of pathogens (Neijenhuis et al. 2001b), thus the risk for IMI.

The increased number of emptying of the udder in treatment cows (both suckling and machine milking) also increases the time for an open teat canal, consequently increasing the risk for invasion of pathogens (Hamann & Osteras 1994). However, the suckling in between milkings which treatment cows experienced, should not have caused additional thickening to the teat wall. Hamann and Stanitzke (1990) reported a decrease in the teat end thickness after suckling, while it increased after milking by machine. What impact suckling had on the teat tissue was not evaluated in the present study, thus, its effect cannot be determined. A potential future research area is how the teats are affected by suckling compared to when milked by machine and its time to recover.

This study presented significant mean increases in TWT of about 46.7% in treatment cows and 58.9% in control cows. Previous studies (Neijenhuis et al. 2001b; Stádník et al. 2010; Martin et al. 2018) reported a thickening of the teat wall post-milking of about 34%, 18% and 40%, respectively. Thus, the increase in TWT was somewhat lower in the previous studies. Overmilking has been reported to cause an even greater increase in TWT (Pařilová et al. 2011; Odorčić et al. 2020). However, the detach level in the present study was 0.5 kg/min, while Martin et al. (2018) used a detach level of 0.3 kg/min in a CMS. Thus, the risk for overmilking was smaller in the present study, so this should not be the reason for thicker teat walls observed in the present study. Almost 50% of the participating cows in the present study were primiparous. Melvin et al. (2019) suggest that the effects of machine milking on the teat tissue is more severe in primiparous cows due to an observed larger relative change in teat canal length compared to cows in higher lactation. Similarly, Rasmussen (1993) observed a significant increased teat end thickness in rear teats of first lactation cows at a detach level of 0.2 kg/min compared to 0.4 kg/min. However, there were no differences in increased teat end diameter at the two detach levels used in front teats and older cows. Whether this explains the higher increase in TWT in the present study is unclear.

Short-term reactions of the teat tissue have been studied in many ways previously (Hamann & Mein 1990; Zecconi et al. 1996; Stádník et al. 2010; Pařilová et al. 2011; Melvin et al. 2019; Odorčić et al. 2020). Some studies have measured the teat canal (Szencziová et al. 2013; Melvin et al. 2019), some the thickness at the teat apex (Hamann & Mein 1990; Zecconi et al. 1992; Hamann et al. 1993; Berglund et al. 2002) or at the barrel (Hamann et al. 1993; Zwertvaegher et al. 2013) and some the teat wall (Stádník et al. 2010; Szencziová et al. 2013). The present study measured the TWT two centimeters above the teat tip. The teat canal length (Szencziová et al. 2013), thus the length of the teat end varies between cows. Yet, according to Weiss et al. (2004) the TWT is correlated to the teat canal length, as the teat canal crosses the teat wall at the teat tip. Therefore, the point of TWT measurement should not be of great importance. On the other hand, Neijenhuis et al. (2001b) discovered that the TWT and TEW changed differently during milking. TEW increased less than the TWT during milking and the recovery time was longer for TEW. The TEW increased less than the TCL (Neijenhuis et al. 2001b), suggesting the teat tip to be more flexible in length than in width.

As for the method used when taking the images with the ultrasound, teats were submerged in a plastic cup filled with lukewarm water. When Bruckmaier and Blum (1992) developed the method for screening udder and teats, they discovered considerable teat contractions when dipped in cold water. The estimation of the temperature in the present study was done with the fingers, therefore the exact temperature could not be determined. Nevertheless, the temperature was probably not sufficiently low to cause contraction.

In some cases, the teats were skewed which made it difficult to get a straight picture of the teat with the ultrasound. To ensure the accuracy in the method the measurements at these occasions could have been repeated about three times and then the mean from these three measurements could have been used.

Regarding the process of sorting out the good quality matched pre- and post-milking images, resulted in 89 erased images, corresponding to about one third of the images. This could give an indication of the difficulty of getting acceptable pictures for future studies.

Whether the increased TWT presented in both treatment cows and control cows in this study could be connected to IMI has not been evaluated. Previous studies have reported that an increased teat thickness > 5% at the apex during milking, as measured with cutimeter, could increase the risk of developing IMI (Zecconi et al. 1996) and a higher proportion of bacterial colonization in the teat duct (Zecconi et al. 1992). The TWT increased approximately 50% in the present study, which could indicate an increased risk of IMI. As mentioned, when discussing TEC, the presented results and whether they affect the udder health could have been strengthened by a comparison to for instance SCC.

As mentioned previously, future research should focus on how the teat tissue respond to suckling compared to machine milking, and if the time to recover differ. This is important for understanding when and for how long the teat canal is open in cow-calf contact systems.

6. Conclusion

Neither the degree of TEC nor TWT change in the present study was higher in treatment cows compared to control cows. The presence of wounds in both treatment cows and control cows was negligible. Thus, the study found no evidence that housing calves in a VMS together with their lactating dams, had negative impact on neither TEC, TWT nor wounds. However, further studies investigating the wearing of teats in cow calf contact systems are needed to verify the results.

References

Ambord, S. & Bruckmaier, R.M. (2010). Milk flow-dependent vacuum loss in high-line milking systems: Effects on milking characteristics and teat tissue condition. *Journal of Dairy Science*, 93 (8), 3588–3594. https://doi.org/10.3168/jds.2010-3059

Barkema, H.W., Deluyker, H.A., Schukken, Y.H. & Lam, T.J.G.M. (1999). Quarter-milk somatic cell count at calving and at the first six milkings after calving. *Preventive Veterinary Medicine*, 38 (1), 1–9. https://doi.org/10.1016/S0167-5877(98)00142-1

Barth, K. (2020). Effects of suckling on milk yield and milk composition of dairy cows in cow–calf contact systems. *Journal of Dairy Research*, 87 (S1), 133–137. https://doi.org/10.1017/S0022029920000515

Berglund, I., Pettersson, G. & Svennersten-Sjaunja, K. (2002). Automatic milking: effects on somatic cell count and teat end-quality. *Livestock Production Science*, 78 (2), 115–124. https://doi.org/10.1016/S0301-6226(02)00090-8

Besier, J. & Bruckmaier, R.M. (2016). Vacuum levels and milk-flow-dependent vacuum drops affect machine milking performance and teat condition in dairy cows. *Journal of Dairy Science*, 99 (4), 3096–3102. https://doi.org/10.3168/jds.2015-10340

Bhutto, A.L., Murray, R.D. & Woldehiwet, Z. (2010). Udder shape and teat-end lesions as potential risk factors for high somatic cell counts and intra-mammary infections in dairy cows. *The Veterinary Journal*, 183 (1), 63–67. https://doi.org/10.1016/j.tvjl.2008.08.024

Breen, J.E., Bradley, A.J. & Green, M.J. (2009a). Quarter and cow risk factors associated with a somatic cell count greater than 199,000 cells per milliliter in United Kingdom dairy cows. *Journal of Dairy Science*, 92 (7), 3106–3115. https://doi.org/10.3168/jds.2008-1562

Breen, J.E., Green, M.J. & Bradley, A.J. (2009b). Quarter and cow risk factors associated with the occurrence of clinical mastitis in dairy cows in the United Kingdom. *Journal of Dairy Science*, 92 (6), 2551–2561. https://doi.org/10.3168/jds.2008-1369

Bruckmaier, R.M. & Blum, J.W. (1992). B-mode ultrasonography of mammary glands of cows, goats and sheep during α- and β-adrenergic agonist and oxytocin administration. *Journal of dairy research*, 59 (2), 151–159. https://doi.org/10.1017/S0022029900030399

Bruckmaier, R.M. & Wellnitz, O. (2008). Induction of milk ejection and milk removal in different production systems. *Journal of Animal Science*, 86 (suppl_13), 15–20. https://doi.org/10.2527/jas.2007-0335

Cardozo, L.L., Thaler Neto, A., Souza, G.N., Picinin, L.C.A., Felipus, N.C., Reche, N.L.M., Schmidt, F.A., Werncke, D. & Simon, E.E. (2015). Risk factors for the occurrence of new and chronic cases of subclinical mastitis in dairy herds in southern Brazil. *Journal of Dairy Science*, 98 (11), 7675–7685. https://doi.org/10.3168/jds.2014-8913

Carlén, E., Strandberg, E. & Roth, A. (2004). Genetic Parameters for Clinical Mastitis, Somatic Cell Score, and Production in the First Three Lactations of Swedish Holstein Cows. *Journal of Dairy Science*, 87 (9), 3062–3070. https://doi.org/10.3168/jds.S0022-0302(04)73439-6

Das, S.M., Redbo, I. & Wiktorsson, H. (2000). Effect of age of calf on suckling behaviour and other behavioural activities of Zebu and crossbred calves during restricted suckling periods. *Applied Animal Behaviour Science*, 67 (1), 47–57. https://doi.org/10.1016/S0168-1591(99)00115-X

De Vliegher, S., Laevens, H., Barkema, H.W., Opsomer, G., Hemling, T. & de Kruif, A. (2003). Short-Term Effect of Transition from Conventional to Automated Milking on Teat Skin and Teat End Condition. *Journal of Dairy Science*, 86 (5), 1646–1652. https://doi.org/10.3168/jds.S0022-0302(03)73750-3

Dohoo, I.R. (1993). An evaluation of the validity of individual cow somatic cell counts from cows in early lactation. *Preventive Veterinary Medicine*, 16 (2), 103–110. https://doi.org/10.1016/0167-5877(93)90080-D

Edwards, J.P., O'Brien, B., Lopez-Villalobos, N. & Jago, J.G. (2013). Overmilking causes deterioration in teat-end condition of dairy cows in late lactation. *Journal of Dairy Research*, 80 (3), 344–348. https://doi.org/10.1017/S0022029913000307

Faul, F., Erdfelder, E., Lang, A.-G. & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, (39), 175–191

Fernández, G., Barreal, M.L., Pombo, M.B., Ginzo-Villamayor, M.J., González-Manteiga, W., Prieto, A., Lago, N. & González-Palencia, J. (2013). Comparison of the epidemiological behavior of mastitis pathogens by applying time-series analysis in results of milk samples submitted for microbiological examination. *Veterinary Research Communications*, 37 (4), 259–267. https://doi.org/10.1007/s11259-013-9570-1

Fernando, R.S. & Spahr, S.L. (1983). Effects of Milking Interval on Selected Milk Constituents from Normal and Infected Quarters1. *Journal of Dairy Science*, 66 (5), 1155–1161. https://doi.org/10.3168/jds.S0022-0302(83)81912-2

Freeman, D.B. (2002). Corns and calluses resulting from mechanical hyperkeratosis. *American Family Physician*, 65 (11), 2277–2280

Fröberg, S., Aspegren-Güldorff, A., Olsson, I., Marin, B., Berg, C., Hernández, C., Galina, C.S., Lidfors, L. & Svennersten-Sjaunja, K. (2007). Effect of restricted suckling on milk yield, milk composition and udder health in cows and behaviour and weight gain in calves, in dual-purpose cattle in the tropics. *Tropical Animal Health and Production*, 39 (1), 71–81. https://doi.org/10.1007/s11250-006-4418-0

Fröberg, S. & Lidfors, L. (2009). Behaviour of dairy calves suckling the dam in a barn with automatic milking or being fed milk substitute from an automatic feeder in a group pen. *Applied Animal Behaviour Science*, 117 (3), 150–158. https://doi.org/10.1016/j.applanim.2008.12.015

Gleeson, D.E., Meaney, W.J., O'Callaghan, E.J. & Rath, M.V. (2004). Effect of teat hyperkeratosis on somatic cell counts of dairy cows. *International Journal of Applied Research in Veterinary Medicine*, 2 (2), 115–122

Grindal, R.J. & Hillerton, J.E. (1991). Influence of milk flow rate on new intramammary infection in dairy cows. *Journal of Dairy Research*, 58 (3), 263–268. https://doi.org/10.1017/S0022029900029848

Guarín, J.F., Paixão, M.G. & Ruegg, P.L. (2017). Association of anatomical characteristics of teats with quarter-level somatic cell count. *Journal of Dairy Science*, 100 (1), 643–652. https://doi.org/10.3168/jds.2016-11459

Guarín, J.F. & Ruegg, P.L. (2016). Short communication: Pre- and postmilking anatomical characteristics of teats and their associations with risk of clinical mastitis in dairy cows. *Journal of Dairy Science*, 99 (10), 8323–8329. https://doi.org/10.3168/jds.2015-10093

Gäde, S., Stamer, E., Bennewitz, J., Junge, W. & Kalm, E. (2007). Genetic parameters for serial, automatically recorded milkability and its relationship to udder health in dairy cattle. *animal*, 1 (6), 787–796. https://doi.org/10.1017/S1751731107000092

de Haas, Y., Barkema, H.W. & Veerkamp, R.F. (2002). The Effect of Pathogen-Specific Clinical Mastitis on the Lactation Curve for Somatic Cell Count. *Journal of Dairy Science*, 85 (5), 1314–1323. https://doi.org/10.3168/jds.S0022-0302(02)74196-9

Halasa, T., Huijps, K., Østerås, O. & Hogeveen, H. (2007). Economic effects of bovine mastitis and mastitis management: A review. *Veterinary Quarterly*, 29 (1), 18–31. https://doi.org/10.1080/01652176.2007.9695224

Hamann, J., Burvenich, C., Mayntz, M., Osteras, O. & Haider, W. (1994). 2. Machine-induced changes in the status of the bovine teat with respect to the new infection risk. *Bulletin of the International Dairy Federation*, (297), 13–22

Hamann, J. & Gyodi, P. (2000). Somatic cells and electrical conductivity in relation to milking frequency. *Milchwissenschaft*, 55, 303–307

Hamann, J. & Mein, G.A. (1990). Measurement of machine-induced changes in thickness of the bovine teat. *Journal of Dairy Research*, 57 (04), 495

Hamann, J., Mein, G.A. & Wetzel, S. (1993). Teat Tissue Reactions to Milking: Effects of Vacuum Level. *Journal of Dairy Science*, 76 (4), 1040–1046. https://doi.org/10.3168/jds.S0022-0302(93)77432-9

Hamann, J. & Osteras, O. (1994). Special aspects. Teat tissue reactions to machine milking and new infection risk. *Bulletin of the International Dairy Federation*, 297, 35–41

Hamann, J. & Stanitzke, U. (1990). Studies on pathogenesis of bovine mastitis by comparison of milking conditions as calf suckling, hand milking and machine milking: reactions of the teat tissue. *Milchwissenschaft*, 45 (10), 632–637

Harmon, R.J. (1994). Physiology of Mastitis and Factors Affecting Somatic Cell Counts1. *Journal of Dairy Science*, 77 (7), 2103–2112. https://doi.org/10.3168/jds.S0022-0302(94)77153-8

Jánosi, Sz. & Baltay, Zs. (2004). Correlations among the somatic cell count of individual bulk milk, result of the California Mastitis Test and bacteriological status of the udder in dairy cows. *Acta Veterinaria Hungarica*, 52 (2), 173–183. https://doi.org/10.1556/avet.52.2004.2.6

Johnsen, J.F., Zipp, K.A., Kälber, T., Passillé, A.M. de, Knierim, U., Barth, K. & Mejdell, C.M. (2016). Is rearing calves with the dam a feasible option for dairy farms?—Current and future research. *Applied Animal Behaviour Science*, 181, 1–11. https://doi.org/10.1016/j.applanim.2015.11.011

Jorstad, A., Farver, T.B. & Riemann, H. (1989). Teat Canal Diameter and Other Cow Factors with Possible Influence on Somatic Cell Counts in Cow Milk. *Acta Veterinaria Scandinavica*, 30 (3), 239–245

Khol, J.L., Franz, S., Klein, D., Lexer, D., Waiblinger, S., Luger, K. & Baumgartner, W. (2006). Influence of milking technique and lactation on the bovine teat by means of ultrasonographic examination. *Berliner und Munchener tierarztliche Wochenschrift*, 119 (1–2), 68–73

Klaas, I.C., Enevoldsen, C., Ersbøll, A.K. & Tölle, U. (2005). Cow-Related Risk Factors for Milk Leakage. *Journal of Dairy Science*, 88 (1), 128–136. https://doi.org/10.3168/jds.S0022-0302(05)72670-9

Luttinen, A. & Juga, J. (1997). Genetic relationships between milk yield, somatic cell count, mastitis, milkability and leakage in Finnish dairy cattle population. *Interbull bulletin*, (15), 78–78

Martin, L.M., Stöcker, C., Sauerwein, H., Büscher, W. & Müller, U. (2018). Evaluation of inner teat morphology by using high-resolution ultrasound: Changes due to milking and establishment of measurement traits of the distal teat canal. *Journal of Dairy Science*, 101 (9), 8417–8428. https://doi.org/10.3168/jds.2018-14500

Mein, F. Neijenhuis, W. F. Morgan, D. J. Reinemann, J. E. Hillerton, J. R. Baines, I. Ohnstad, M. D. Rasmussen, L. Timms, J. S. Britt, R. Farnsworth, N. Cook, & T. Hemling (2001). *Evaluation of Bovine Teat Condition in Commercial Dairy Herds: 1. Non-Infectious Factors*.

Melvin, J.M., Heuwieser, W., Virkler, P.D., Nydam, D.V. & Wieland, M. (2019). Machine milking–induced changes in teat canal dimensions as assessed by ultrasonography. *Journal of Dairy Science*, 102 (3), 2657–2669. https://doi.org/10.3168/jds.2018-14968

Mendoza, A., Cavestany, D., Ariztia, J., Pereira, C., La Manna, A., Contreras, D.A. & Galina, C.S. (2010). Effect of restricted suckling on milk yield, composition and flow, udder health, and postpartum anoestrus in grazing Holstein cows. *Livestock Science*, 127 (1), 60–66. https://doi.org/10.1016/j.livsci.2009.08.006

Natzke, R.P., Everett, R.W. & Bray, D.R. (1982). Effect of Overmilking on Udder Health1. *Journal of Dairy Science*, 65 (1), 117–125. https://doi.org/10.3168/jds.S0022-0302(82)82160-7

Neijenhuis, F., Barkema, H.W., Hogeveen, H. & Noordhuizen, J.P.T.M. (2000). Classification and Longitudinal Examination of Callused Teat Ends in Dairy Cows. *Journal of Dairy Science*, 83 (12), 2795–2804. https://doi.org/10.3168/jds.S0022-0302(00)75177-0

Neijenhuis, F., De Koning, H., Barkema, H. & Hogeveen, H. (2001a). The effects of machine milking on teat condition. *ICAR Technical series*, (7), 33–40

Neijenhuis, F., Klungel, G.H. & Hogeveen, H. (2001b). Recovery of Cow Teats after Milking as Determined by Ultrasonographic Scanning. *Journal of Dairy Science*, 84 (12), 2599–2606. https://doi.org/10.3168/jds.S0022-0302(01)74714-5

Odorčić, M., Blau, U., Löfstrand, J. & Bruckmaier, R.M. (2020). Short communication: Teat wall diameter and teat tissue thickness in dairy cows are affected by intramammary pressure and by the mechanical forces of machine milking. *Journal of Dairy Science*, 103 (1), 884–889. https://doi.org/10.3168/jds.2019-16565

Olde Riekerink, R.G.M., Barkema, H.W., Veenstra, W., Berg, F.E., Stryhn, H. & Zadoks, R.N. (2007). Somatic Cell Count During and Between Milkings. *Journal of Dairy Science*, 90 (8), 3733–3741. https://doi.org/10.3168/jds.2007-0001

Paduch, J.-H., Mohr, E. & Krömker, V. (2012). The association between teat end hyperkeratosis and teat canal microbial load in lactating dairy cattle. *Veterinary Microbiology*, 158 (3), 353–359. https://doi.org/10.1016/j.vetmic.2012.02.032

Pantoja, J.C.F., Almeida, A.P., dos Santos, B. & Rossi, R.S. (2016). An investigation of risk factors for two successive cases of clinical mastitis in the same lactation. *Livestock Science*, 194, 10–16. https://doi.org/10.1016/j.livsci.2016.10.010

Pantoja, J.C.F., Correia, L.B.N., Rossi, R.S. & Latosinski, G.S. (2020). Association between teat-end hyperkeratosis and mastitis in dairy cows: A systematic review. *Journal of Dairy Science*, 103 (2), 1843–1855. https://doi.org/10.3168/jds.2019-16811

Pařilová, M., Stádník, L., Ježková, A. & Štolc, L. (2011). Effect of milking vacuum level and overmilking on cows' teat characteristics. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 59 (5), 193–202. https://doi.org/10.11118/actaun201159050193
de Passillé, A.M., Marnet, P.-G., Lapierre, H. & Rushen, J. (2008). Effects of Twice-Daily Nursing on Milk Ejection and Milk Yield During Nursing and Milking in Dairy Cows. *Journal of Dairy Science*, 91 (4), 1416–1422. https://doi.org/10.3168/jds.2007-0504
Paulrud, C.O. (2005). Basic Concepts of the Bovine Teat Canal. *Veterinary Research Communications*, 29 (3), 215–245. https://doi.org/10.1023/B:VERC.0000047496.47571.41
Penry, J.F., Endres, E.L., de Bruijn, B., Kleinhans, A., Crump, P.M., Reinemann, D.J. & Hernandez, L.L. (2017a). Effect of incomplete milking on milk production rate and composition with 2 daily milkings. *Journal of Dairy Science*, 100 (2), 1535–1540. https://doi.org/10.3168/jds.2016-11935
Penry, J.F., Upton, J., Mein, G.A., Rasmussen, M.D., Ohnstad, I., Thompson, P.D. & Reinemann, D.J. (2017b). Estimating teat canal cross-sectional area to determine the effects of teat-end and mouthpiece chamber vacuum on teat congestion. *Journal of Dairy Science*, 100 (1), 821–827. https://doi.org/10.3168/jds.2016-11533
Persson, Y., Nyman, A.-K.J. & Grönlund-Andersson, U. (2011). Etiology and antimicrobial susceptibility of udder pathogens from cases of subclinical mastitis in dairy cows in Sweden. *Acta Veterinaria Scandinavica*, 53 (1), 36. https://doi.org/10.1186/1751-0147-53-36
de Pinho Manzi, M., Nóbrega, D.B., Faccioli, P.Y., Troncarelli, M.Z., Menozzi, B.D. & Langoni, H. (2012). Relationship between teat-end condition, udder cleanliness and bovine subclinical mastitis. *Research in Veterinary Science*, 93 (1), 430–434. https://doi.org/10.1016/j.rvsc.2011.05.010
Plummer, P.J. & Plummer, C. (2012). Chapter 15 - Diseases of the Mammary Gland. I: Pugh, D.G. & Baird, A.N. (red.) *Sheep and Goat Medicine (Second Edition)*. Saint Louis: W.B. Saunders, 442–465. https://doi.org/10.1016/B978-1-4377-2353-3.10015-0
Rasmussen, M. (2004). Overmilking and teat condition. *NMC Annual Meeting Proceedings*,
Rasmussen, M.D. (1993). Influence of switch level of automatic cluster removers on milking performance and udder health. *Journal of Dairy Research*, (60), 287–297
Rasmussen, M.D. & Larsen, H.D. (1998). The Effect of Post Milking Teat Dip and Suckling on Teat Skin Condition, Bacterial Colonisation, and Udder Health. *Acta Veterinaria Scandinavica*, 39 (4), 443–452
Sandrucci, A., Bava, L., Tamburini, A. & Zanini, L. (2005). Milking procedures, milk flow curves and somatic cell count in dairy cows. *Italian Journal of Animal Science*, 4 (sup2), 215–217. https://doi.org/10.4081/ijas.2005.2s.215
Sandrucci, A., Bava, L., Zucali, M., Tamburini, A., Sandrucci, A., Bava, L., Zucali, M. & Tamburini, A. (2014). Management factors and cow traits influencing milk somatic cell counts and teat hyperkeratosis during different seasons. *Revista Brasileira de Zootecnia*, 43 (9), 505–511. https://doi.org/10.1590/S1516-35982014000900008
Sandrucci, A., Tamburini, A., Bava, L. & Zucali, M. (2007). Factors Affecting Milk Flow Traits in Dairy Cows: Results of a Field Study. *Journal of Dairy Science*, 90 (3), 1159–1167. https://doi.org/10.3168/jds.S0022-0302(07)71602-8
Sargeant, J.M., Leslie, K.E., Shirley, J.E., Pulkrabek, B.J. & Lim, G.H. (2001). Sensitivity and Specificity of Somatic Cell Count and California Mastitis

Test for Identifying Intramammary Infection in Early Lactation1. *Journal of Dairy Science*, 84 (9), 2018–2024. https://doi.org/10.3168/jds.S0022-0302(01)74645-0
Schepers, A.J., Lam, T.J.G.M., Schukken, Y.H., Wilmink, J.B.M. & Hanekamp, W.J.A. (1997). Estimation of Variance Components for Somatic Cell Counts to Determine Thresholds for Uninfected Quarters. *Journal of Dairy Science*, 80 (8), 1833–1840. https://doi.org/10.3168/jds.S0022-0302(97)76118-6
Schukken, Y.H., Wilson, D.J., Welcome, F., Garrison-Tikofsky, L. & Gonzalez, R.N. (2003). Monitoring udder health and milk quality using somatic cell counts. *Veterinary Research*, 34 (5), 579–596. https://doi.org/10.1051/vetres:2003028
Shearn, M.F.H. & Hillerton, J.E. (1996). Hyperkeratosis of the teat duct orifice in the dairy cow. *Journal of Dairy Research*, 63 (4), 525–532. https://doi.org/10.1017/S0022029900032064
Sheldrake, R.F., Hoare, R.J.T. & McGregor, G.D. (1983). Lactation Stage, Parity, and Infection Affecting Somatic Cells, Electrical Conductivity, and Serum Albumin in Milk. *Journal of Dairy Science*, 66 (3), 542–547. https://doi.org/10.3168/jds.S0022-0302(83)81823-2
Sirovnik, J., Barth, K., Oliveira, D. de, Ferneborg, S., Haskell, M.J., Hillmann, E., Jensen, M.B., Mejdell, C.M., Napolitano, F., Vaarst, M., Verwer, C.M., Waiblinger, S., Zipp, K.A. & Johnsen, J.F. (2020). Methodological terminology and definitions for research and discussion of cow-calf contact systems. *Journal of Dairy Research*, 87 (S1), 108–114. https://doi.org/10.1017/S0022029920000564
Stádník, L., Louda, F., Bezdíček, J., Ježková, A. & Rákos, M. (2010). Changes in teat parameters caused by milking and their recovery to their initial size. *Archiv Tierzucht*, 53 (6), 650–662. https://www.cabdirect.org/cabdirect/abstract/20113117693 [2020-05-25]
Strapák, P., Szencziová, I. & Strapáková, E. (2018). Measurement of teat structures o dairy cows through ultrasonography and examination of morphological changes in teats caused by machine milking. *Veterinarija ir Zootechnika*, 76 (98)
Szencziová, I., Strapák, P., Stádník, L., Ducháček, J. & Beran, J. (2013). Relationship of Udder and Teat Morphology to Milking Characteristics and Udder Health Determined by Ultrasonographic Examinations in Dairy Cows / Związek Między Morfologią Wymienia I Strzyków A Cechami Doju I Zdrowotnością Wymienia W Badaniach Usg Krów Mlecznych. *Annals of Animal Science*, 13 (4), 783–795. https://doi.org/10.2478/aoas-2013-0053
Tančin, V. & Bruckmaier, R.M. (2015). Factors affecting milk ejection and removal during milking and suckling of dairy cows. *Veterinární Medicína*, 46 (No. 4), 108–118. https://doi.org/10.17221/7860-VETMED
Tančin, V., Ipema, B., Hogewerf, P. & Mačuhová, J. (2006). Sources of Variation in Milk Flow Characteristics at Udder and Quarter Levels. *Journal of Dairy Science*, 89 (3), 978–988. https://doi.org/10.3168/jds.S0022-0302(06)72163-4
Tancin, V., Kraetzl, W.-D., Schams, D. & Bruckmaier, R.M. (2001). The effects of conditioning to suckling, milking and of calf presence on the release of oxytocin in dairy cows. *Applied Animal Behaviour Science*, 72 (3), 235–246. https://doi.org/10.1016/S0168-1591(01)00113-7
Tsenkova, R., Atanassova, S., Kawano, S. & Toyoda, K. (2001). Somatic cell count determination in cow's milk by near-infrared spectroscopy: a new diagnostic tool. *Journal of Animal Science*, 79 (10), 2550. https://doi.org/10.2527/2001.79102550x

Wall, S.K., Wellnitz, O., Bruckmaier, R.M. & Schwarz, D. (2018). Differential somatic cell count in milk before, during, and after lipopolysaccharide- and lipoteichoic-acid-induced mastitis in dairy cows. *Journal of Dairy Science*, 101 (6), 5362–5373. https://doi.org/10.3168/jds.2017-14152

Weiss, D., Weinfurtner, M. & Bruckmaier, R.M. (2004). Teat Anatomy and its Relationship with Quarter and Udder Milk Flow Characteristics in Dairy Cows. *Journal of Dairy Science*, 87 (10), 3280–3289. https://doi.org/10.3168/jds.S0022-0302(04)73464-5

Wieland, M., Nydam, D.V. & Virkler, P.D. (2017). A longitudinal field study investigating the association between teat-end shape and two minute milk yield, milking unit-on time, and time in low flow rate. *Livestock Science*, 205, 88–97. https://doi.org/10.1016/j.livsci.2017.09.011

Wieland, M., Virkler, P.D., Borkowski, A.H., Älveby, N., Wood, P. & Nydam, D.V. (2019). An observational study investigating the association of ultrasonographically assessed machine milking-induced changes in teat condition and teat-end shape in dairy cows. *animal*, 13 (2), 341–348. https://doi.org/10.1017/S1751731118001246

Zadoks, R.N., Allore, H.G., Barkema, H.W., Sampimon, O.C., Wellenberg, G.J., Gröhn, Y.T. & Schukken, Y.H. (2001). Cow- and Quarter-Level Risk Factors for Streptococcus uberis and Staphylococcus aureus Mastitis. *Journal of Dairy Science*, 84 (12), 2649–2663. https://doi.org/10.3168/jds.S0022-0302(01)74719-4

Zecconi, A., Bronzo, V., Piccinini, R., Moroni, P. & Ruffo, G. (1996). Field study on the relationship between teat thickness changes and intramammary infections. *Journal of Dairy Research*, 63 (3), 361–368. https://doi.org/10.1017/S0022029900031885

Zecconi, A., Hamann, J., Bronzo, V., Moroni, P., Giovannini, G. & Piccinini, R. (2006). Relationship Between Teat Tissue Immune Defences and Intramammary Infections. 287–293. https://doi.org/10.1007/0-306-46832-8_33

Zecconi, A., Hamann, J., Bronzo, V. & Ruffo, G. (1992). Machine-induced teat tissue reactions and infection risk in a dairy herd free from contagious mastitis pathogens. *Journal of Dairy Research*, 59 (3), 265–271. https://doi.org/10.1017/S0022029900030545

Zhao, X. & Lacasse, P. (2008). Mammary tissue damage during bovine mastitis: Causes and control1. *Journal of Animal Science*, 86 (suppl_13), 57–65. https://doi.org/10.2527/jas.2007-0302

Zoche-Golob, V., Haverkamp, H., Paduch, J.-H., Klocke, D., Zinke, C., Hoedemaker, M., Heuwieser, W. & Krömker, V. (2015). Longitudinal study of the effects of teat condition on the risk of new intramammary infections in dairy cows. *Journal of Dairy Science*, 98 (2), 910–917. https://doi.org/10.3168/jds.2014-8446

Zwertvaegher, I., De Vliegher, S., Verbist, B., Van Nuffel, A., Baert, J. & Van Weyenberg, S. (2013). Short communication: Associations between teat dimensions and milking-induced changes in teat dimensions and quarter milk somatic cell counts in dairy cows. *Journal of Dairy Science*, 96 (2), 1075–1080. https://doi.org/10.3168/jds.2012-5636