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Effect of machine milking on bovine teat sinus injury and teat canal keratin

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A study was undertaken to establish, through morphological analysis, changes in teat cellular structure which may occur as a result of machine milking with different machine settings. Two milking machine systems were compared in two experiments. There were two treatments, namely, NB with 1.65kg clusters, 275ml claws, narrow-bore tapered liners (25.0mm – 20.0mm) with 2x2 pulsation, and WB with 3.2kg clusters, 150ml claws, wide-bore tapered liners (31.6mm – 20.6mm) and 4x0 pulsation. Cows were randomised into groups for Experiment 1 based on milk yield, cell count and teat-end condition; they were randomised in Experiment 2 based on lactation number. In Experiment 1, eight cows were assigned to either WB or NB milking systems for 118 days at the end of lactation. Clusters were removed at a milk flow-rate of 0.2 kilograms per minute or after five minutes of over-milking. In Experiment 2, eighteen cows were assigned to either WB or NB systems post-calving, and were milked for a complete lactation. Three cows from each milking system were subsequently allowed a 42-day dry period before culling. The cows in both experiments were slaughtered and their teats were recovered and fixed in a formalin solution within three hours of their last milking, or after the 42-day dry period. Sections of teat tissue were examined under a light microscope and assessed for teat sinus morphology. The teat sinus was visually scored from zero to five for epithelial damage, increased red blood cells and oedema. The total score for each teat was then expressed as total teat sinus injury (TSI). The area of the teat canal epithelium and the area of keratin were measured and the amount of teat canal keratin (TCK) was expressed as a percentage of the overall area. There was not a difference in TCK between the milking systems, when first lactation cows were compared. There were lower ($p<0.05$) oedema scores with the NB milking system than with the WB system in Experiments 1 and 2. Teats over-milked for five minutes had higher ($p<0.001$) TSI and lower ($p<0.01$) TCK than teats from which clusters were removed at a milk flow-rate of 0.2kg/min. The areas of TCK as a percentage of the teat canal epithelium was greater ($p<0.001$) in first lactation cows than in cows with four or more lactations completed. Front and hind teats had similar results for each teat parameter measured. TCK increased ($p<0.05$) following a 42-day dry period, but TSI was not significantly better.

Keywords
Cattle,
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Introduction

In Ireland, cows are generally milked with milking machines with 3.2kg clusters, with wide-bore tapered liners (31.6mm – 20.6mm) and 4x0 pulsation (WB). Outside of Ireland, most milking machines have 1.65kg clusters, narrow-bore liners (25.0mm – 20.0mm) and 2x2 pulsation (NB). O'Callaghan (2001) showed that the mouthpiece cavity vacuum was higher with wide-bore tapered liners than with narrow bore peer reviewed

liners. O'Callaghan (1997) also showed that the teat-end vacuum during the milking phase with wide-bore tapered liners used with 4x0 pulsation was 7kPa higher than that with narrow-bore liners used with 2x2 pulsation. Teat exposure to this increased vacuum may result in increased levels of teat sinus injury. Vacuum within an empty teat sinus has been shown to equal that at the teat-end (Witzel, 1965). Mechanical forces applied with different milking units were also shown to result in different ratios of cell types in teat end tissues (Bronzo *et al.*, 1995). A properly functioning milking machine, when used incorrectly, is capable of severely injuring delicate internal teat structures. A study by Peterson (1964) showed that over-milking of teats by twenty minutes for four milkings resulted in increased pathological changes including hyperaemia, haemorrhage, and oedema of the sub-epithelial tissues. This effect may be increased or decreased depending on the milking system used. In theory, milk within the teat acts as a cushion against the collapse of the teat-cup liner and prevents frictional irritation to the epithelial lining of the teat sinus and glands. The expanding and collapsing action of teat-cup liners on empty teats was shown to be injurious to teat tissue (Pier *et al.*, 1956). In milking parlours where automatic cluster removal is not installed there is some level of over-milking. Extended machine milking times were also shown to be potentially damaging to mammary tissue (Dodd *et al.*, 1950). The presence of keratin appears to have an important protective function against bacterial ingress through the canal. Murphy (1959) concluded that removal of a small amount of soft, waxy keratin lining the lumen of a resistant teat canal rendered the gland susceptible to an experimental bacterial challenge. The physical properties of keratin do not appear to influence the susceptibility to a bacterial challenge (Treece *et al.*, 1966). The amount of keratin present in the teat canal may be more important as a defence mechanism than the physical properties of keratin. Milking with pulsation removes up to 40% of the keratin that was present before milking; removal is caused by the shear forces associated with milk flow (Capuco *et al.*, 1990). The layers of keratin can adsorb bacteria, and any milking process that greatly increases the amount of keratin removed during milking may increase the probability of mastitis infection. It was suggested that pulsation action protects the teat from excessive loss of keratin (Williams and Mein, 1980). However, over-milking may also result in the removal of excessive amounts of teat canal keratin (McDonald, 1975). Machine settings associated with different milking systems influence the physical properties of teat canal keratin (Hamann, 1987). Lacy-Hulbert (1998) suggested that plant vacuum level did not affect keratin loss.

The objective of the present study was to determine the effect of two milking systems and over-milking for five minutes on teat sinus injury (TSI) and teat canal keratin (TCK) and also to quantify teat recovery during the dry period.

Materials and methods

Experiment 1

Eight Holstein-Friesian type dairy cows were randomised to one of four groups based on teat condition, somatic cell count and milk yield. The groups were then assigned to four milking treatments for 118 days in late lactation. Treatment 1 (NB) was a milking system with 1.65kg clusters, 275ml claws, narrow-bore tapered liners (25mm – 20mm) and alternate pulsation. Treatment 3 (WB) had 3.2kg clusters, 150ml claws, wide-bore tapered liners (31.6mm – 20.6mm) and simultaneous pulsation. Clusters were automatically removed when milk flow-rate dropped to 0.2kg/min in Treatments 1 and 3. Treatments 2 and 4 consisted of the NB and WB milking systems, respectively, with clusters automatically removed 5 minutes after milk flow-rate dropped to 0.2kg/min. The phases of the pulsation chamber waveform for NB and WB, respectively, were: a = 18.3, 17.0; b = 46.8, 51.4; c = 11.5, 11.8; and d = 23.4, 19.8. Pulsation rate was 60 cycles/min and the plant effective vacuum reserve was 1,540 litres/min for both milking systems. All cows were milked in an 80-degree side-by-side milking parlour, with a milk lift of 1.2m from the cow standing to a 72-mm id milkline. Cows were slaughtered and the teats harvested and stored in a solution of neutral formalin within three hours of their last milking. Sections of teat tissue were examined and assessed for teat sinus morphology.

Experiment 2

Eighteen Holstein-Friesian type dairy cows were randomised to two groups and milked with either WB or NB milking systems post-calving; randomisation was based on lactation number, teat condition, somatic cell count and milk yield. All cows were milked for a complete lactation in the same milking facility and pulsation settings used for Experiment 1. Cows were milked at intervals of 17h (overnight) and 7h (daytime). Pre-milking teat preparation consisted of washing with running water and drying with paper towels. All milking clusters were automatically removed when milk flow-rate dropped to 0.2kg/min. Teats were disinfected post-milking with a chlorohexidine-based teat disinfectant. At the end of lactation, six first lactation cows from each milking treatment were culled within three hours of their last milking (T1 and T2). Milking of the remaining three cows in each treatment ceased at the same time and they received dry cow antibiotic therapy (T3 and T4). These six cows, with lactation numbers ranging from two to eight, were allowed a six-week dry period before culling. All teats were harvested and stored in a solution of neutral formalin. Sections of teat tissue were examined under a light microscope and teat sinuses were assessed morphologically.

Histological examination of teat specimens

The formalin-fixed blocks of tissue were routinely processed into paraffin wax. Transverse sections through the full thickness

TABLE 1: Effect of milking system design and over-milking on injury to the teat epithelial lining, extravasation of red blood cells, oedema and teat canal keratin - Experiment 1

		Narrow bore T1 0.2kg/min	Narrow bore T2 0.2kg/min+5min	Wide bore T3 0.2kg/min	Wide bore T4 0.2kg/min+5min
Epithelial damage	Mean	1.13	2.25	1.5	2.63
	Min/Max	1/2	2/4	1/2	2/4
	Median	1^{a}	2^{b}	1.5^{a}	2.5^{b}
Red blood cells	Mean	0.3	0.75	0.5	3.0
	Min/Max	0/1	0/2	0/2	1/4
	Median	0^{a}	0.5^{a}	0^{a}	3^{b}
Oedema	Mean	0.38	1.25	1.42	1.5
	Min/Max	0/1	0/4	1/3	1/4
	Median	0^{a}	1^{a}	1^{c}	1^{c}
Total sinus injury	Mean	1.63	4.25	3.42	7.13
	Min/Max	1/3	2/7	2/7	6/10
	Median	1.5^{a}	4^{b}	3^{b}	7^{b}
Teat canal keratin	Mean	0.85^{a}	0.99^{a}	1.24^{ab}	0.65^{a} s.e.d. (0.05)
	Area %	27.6	29.7	27.7	25.1 s.e.d. (0.50)

Values with different superscripts, within rows, within cluster groups, differ significantly, ^{ab} p<0.001, ^{ac} P<0.05. Number of teats = 8 per treatment.

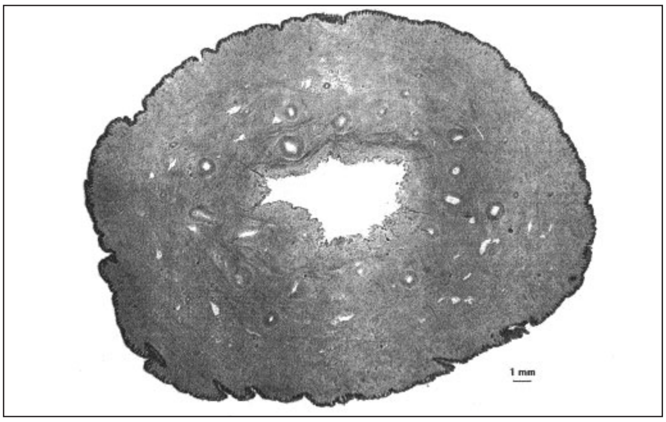

FIGURE 1: Full thickness cross-section through the teat at the level of the teat sinus.

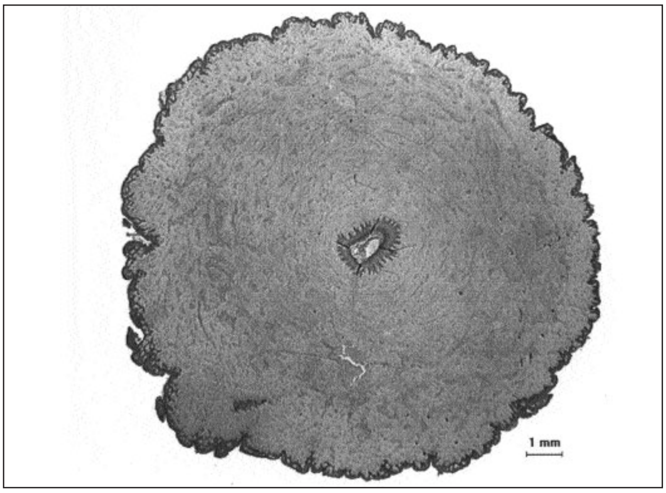

FIGURE 2: Full thickness cross-section through the teat at the level of the teat canal.

of the teat were cut, stained with haematoxylin and eosin and examined under a light microscope. Sections across the teat sinus (Figure 1) were assessed for teat sinus injury (TSI): abnormalities of the epithelial lining, the presence of oedema and extravasation of red blood cells from the sub-epithelial blood vessels. The indivdual changes noted in each sample were visually graded on a scale from zero to five depending on the degree of tissue change, five indicating most damage. The total score for each teat was then expressed as total teat sinus injury (TSI).
Sections taken across the teat canal (Figure 2) were used to calculate the area of teat canal epithelium (mm^2) and the area of keratin (mm^2), and the amount of keratin was expressed as a percentage of the total area.
One operator, without knowledge of the treatments, carried out the grading for TSI and measurements of TCK.
TSI and TCK values were compared for the two milking systems, for over-milked teats versus teats after normal cluster removal, and for teats after a dry period of 42 days versus teats at drying off. Front teats were compared to rear teats, and first lactation cows were compared to older cows for TSI and TCK in Experiment 1 only.

Statistical analysis

Procedures of the Statistical Analyses System (SAS, 1989) were used to analyse the data. The individual teat was the experimental unit for analysis of all data. Non-parametric teat scores for the variables (epithelium, red blood cell, oedema and total sinus score) were analysed by the one-way analysis of variance (ANOVA) of Kruskal and Wallis (Conover, 1980) using the PROC GLM procedure on ranked data. Treatment medians for epithelium, oedema, red blood cells and total sinus score were compared using the Least Significant Difference procedure and are presented in Tables 1 to 3. A Mann-Whitney U test was performed to detect differences in the effect of cluster type, over-milking and front versus rear teats. Treatment means were used to present keratin as a percentage of the epithelial lining and the canal lumen. Data for variables keratin and keratin area were not ranked and were analysed by the one-way ANOVA with a Tukey Studentized Range test where appropriate. A t-test was used to detect the effect of cluster type, over-milking and front versus hind teats on keratin and keratin area.

Results

Experiment 1

The effects of milking system and over-milking on TSI and

TABLE 2: Effect of milking system design and a 42-day dry period on condition of the teat epithelial lining, extravasation of red blood cells, oedema and teat canal keratin - Experiment 2

		Wide bore T1	Narrow bore T2	Wide bore T3 dry teats	Narrow bore T4 dry teats
Epithelial damage	Mean	2.27	2.2	2.75	2.83
	Min/Max	1/5	1/4	1/4	2/4
	Median	2	2	3	3
Red blood cells	Mean	1.77	1.79	1.92	1.58
	Min/Max	1/5	1/4	1/4	1/3
	Median	1	1	1	1
Oedema	Mean	1.68	1.0	1.16	1.33
	Min/Max	1/5	1/1	1/3	1/3
	Median	1^{a}	1^{b}	1	1
Total sinus injury	Mean	5.7	5.0	5.8	5.8
	Min/Max	3/15	3/9	3/9	4/8
	Median	4	4	5	5.5
Teat canal keratin	Mean	0.75^{a}	0.64^{a}	1.09^{c}	0.77^{a}
	Area %	23.2	21.7^{b}	29.5^{a}	26.4

Values with different superscripts, within rows, within cluster groups, differ significantly, $^{ac}p<0.01$, $^{ab}P<0.05$. Number of teats = 8 per treatment.

TABLE 3: Effect of teat position on damage to the teat epithelial lining, extravasation of red blood cells, oedema and teat canal keratin - Experiments 1 and 2

		EXPERIMENT 1		EXPERIMENT 2	
		Front teats	Rear teats	Front teats	Rear teats
Epithelial damage	Mean	2.44	2.42	2.51	2.33
	Min/Max	1/5	1/4	1/5	1/5
	Median	2	2	3	2
Red blood cells	Mean	1.82	1.72	1.95	1.58
	Min/Max	1/5	1/4	1/5	1/5
	Median	1	1	1	1
Oedema	Mean	1.5	1.11	1.30	1.30
	Min/Max	1/5	1/3	1/4	1/5
	Median	1	1	1	1
Total sinus injury	Mean	5.76	5.25	5.76	5.21
	Min/Max	3/15	3/9	3/14	3/15
	Median	4	4.5	5	4
Teat canal keratin	Mean	0.86	0.62	0.73	0.74 s.e.d. (0.07)
	Area %	25.3	23.3	24.5	24.1 s.e.d. (1.0)

Number of teats = 16 per treatment.

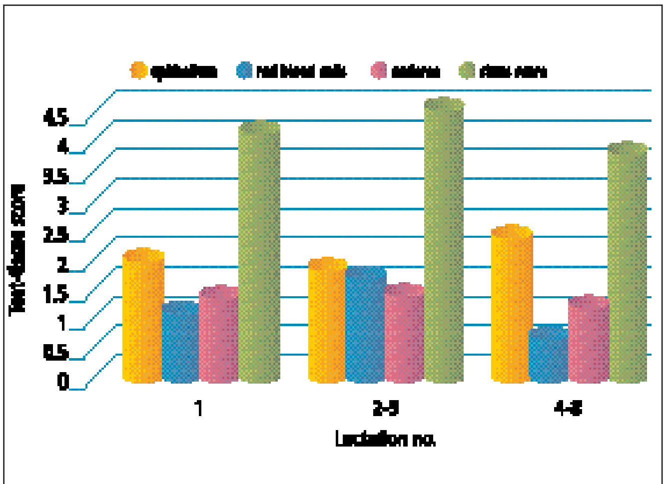

FIGURE 3: Effect of lactation number on mean injury to the epithelium, the presence of red blood cells, oedema and total sinus score.

TCK are shown in Table 1. Teats over-milked for five minutes had a higher ($p<0.001$) level of damage to the epithelial lining, more ($p<0.001$) extravasation of red blood cells and a higher ($p<0.001$) TSI score than teats with clusters removed at a milk flow-rate of 0.2kg/min. Levels of TCK were lower ($p<0.01$) in over-milked teats. Treatment 4 (WB over-milked) had higher ($p<0.001$) extravasation of red blood cells and lower ($p<0.001$) levels of TCK than T3 (WB with early cluster removal). Treatment 2 (NB over-milked) had higher ($p<0.001$) levels of damage to the epithelial lining than Treatment 1 (NB with early cluster removal). Treatment 1 also had a lower ($p<0.05$) score for oedema than T3 and T4. The area of keratin as a percentage of the epithelial lining was similar for all treatments. TSI and TCK values for front and rear teats did not differ significantly (Table 3). The mean TSI scores (Figure 3) were 4.0 for teats with one lactation completed (12 teats), 4.3 for teats with two or three lactations completed (12 teats) and 3.6 for teats with four to eight lactations completed (eight teats). There was not a significant difference in damage to the epithelium lining the teat sinus, presence of red blood cells, or oedema between teats with different lactation numbers completed. For the same groups of teats, the measurements for TCK were 0.97, 0.96, 1.0, respectively, and for areas of TCK were 30.2%, 25.8% and 25.4%, respectively. First lactation cows had a higher ($p<0.001$) area of TCK as a percentage of the epithelial lining as compared with cows with two or more lactations completed.

Experiment 2

The effects of two milking systems and a 42-day dry period on TSI and TCK are shown in Table 2. There was not a significant difference in TSI levels between teats harvested after a 42-day dry period and teats harvested within three hours of their last milking. Treatment 2 had a lower ($p<0.05$) score for oedema than T1. Treatment 3 had a higher ($p<0.01$) TCK score than T1, T2 and T4. Treatments 3 and 4 (dry teats) had a higher ($p<0.05$) level of keratin expressed as a percentage of the epithelial lining than T1 and T2. There was not a significant difference in TSI and TCK between front and rear teats (Table 3).

Discussion

Over-milking of teats for five minutes at each milking over a four-month period resulted in significantly more TSI and a greater loss of TCK than cluster removal at a milk flow-rate of 0.2kg/min. These results agree with the findings of Peterson (1964), in that over-milking caused teat tissue damage, and also agree with the findings of McDonald (1975), in that over-milking reduced keratin. The main teat sinus injury was to the epithelial lining, which had increased red blood cell numbers. There were some differences in TSI with increasing lactation number. This may explain the differences between milking systems for TSI in Experiment 1, as cows were not assigned to treatments based on lactation number. There was not a significant difference between WB and NB in the level of epithelial injury, extravasation of red blood cells and TSI when first lactation cows were compared in Experiment 2. The epithelial layers which surround the teat canal increase in thickness with increasing age of cows (Michel *et al*., 1974); this may explain why heifers had a higher percentage of teat canal keratin as a percentage of the area of the epithelial lining and canal lumen combined. In a separate investigation, teats that had not been milked for 112 days had low TSI and high TCK levels (Gleeson, unpublished results). This may indicate that during an extended dry period teats would recover from the trauma caused by machine milking. However, teats harvested in the present study following a 42-day dry period had the same TSI level as teats harvested within three hours of their last milking. The fact that the cows that had the dry period had completed more than one lactation may explain why the TSI had not improved. Alternatively, this result may indicate that the non-lactating period should extend beyond 42 days to allow full teat tissue recovery. After a 42-day dry period teats had a higher level of keratin, expressed as a percentage of the epithelial lining, than lactating teats. This indicates that discontinuation of milking can increase the amount of keratin in the teat canal. Front teats produce less milk than rear teats and, therefore, some over-milking occurs in front teats during normal milking. However, when front teats were compared to rear teats, there was not a significant difference in any teat parameters measured, including quantity and percentage of keratin.

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