1 INTRODUCTION

Along with the continuous regeneration of bovine teat canal lining to replace the keratinized superficial strata of the epithelium, tissues ensuring the proper closing of the Fürstenberg's rosette play a major role in the prevention of both milk leakage and the entry of bacteria into the teat sinus. Knowledge of good teat structure may help breed more healthy animals and reduce mastitis and the development of antimicrobial resistance (Aslantas & Demir, 2016).

The possible connection between teat morphology and mastitis has been studied extensively (Guarin & Ruegg, 2016; Singh, Bansal, & Gupta, 2017; Slettbakk, Jørstad, Farver, & Holmes, 1995), but detailed histological studies of various tissue components in the teat canal are few (Ozenc et al., 2020; Pounden & Grossman, 1950; Venzke, 1940). Some previous studies (Giesecke, Gerneke, & van Rensburg, 1972; Paulrud, 2005; Pounden & Grossman, 1950; Van der Merwe, 1985) suggest that teat canal closure depends primarily on passive elastic contractions augmented by the tone of the smooth muscle fibres, while Hamann and Burvenich (1994) described the closure as an active process related to the spirally distributed smooth muscle in the teat wall. Regardless of the closing mechanism, teat canal keratin helps block the teat canal during the dry season. The objective of our study was to estimate the amount of collagen and elastic tissues surrounding the Fürstenberg's rosette in mastitis-infected and non-infected cows.

2 MATERIALS AND METHODS

Tissue types were analysed from 19 milking Ayrshire and Holstein cows slaughtered at a single Finnish slaughterhouse. The examined cows were part of a larger data set that was previously used to characterize teat morphology with 3D imaging (Vesterinen et al., 2015). During the analyses, certain samples were excluded due to various technical or tissue quality problems (foldings, tears) apparent only after completion of the histological process. Finally, the collagen fibre content was estimated from 16 teats from cows that had had mastitis or were slaughtered because of mastitis and from 46 teats.
from cows with no history of mastitis. Corresponding numbers for the elastic fibre analyses were 13 teats from cows that had had mastitis or were slaughtered because of mastitis and 36 teats from cows with no history of mastitis. Most of the examined histological samples were taken from Ayrshire cows (44/49 for elastic fibres and 56/62 for collagen fibres).

### 2.1 Preparation and analysis of histological samples

Standard histological transverse sections (4 \( \mu m \)) of teat tissue at the level of the Fürstenberg’s rosette were stained with Van Gieson (for connective tissue) and aldehyde fuchsin (for elastic fibres). 100 x magnification was used for examining collagen fibres, to ensure as large a field of view as possible without compromising good resolution without artefacts. For the examination of elastic fibres, 400 x magnification was needed for sufficient resolution. Because fibres were unevenly distributed in the tissues, four microscopic fields of each teat (around the Fürstenberg’s rosette just inside the epithelium) and each staining were photographed (with automatic settings) and analysed using ImageJ software ([imagej.nih.gov/ij/download/](http://imagej.nih.gov/ij/download/)) to quantify the amount of tissue area that consisted of collagen and elastic fibres. The programme figure type was RGB stack, and green was chosen from the colour alternatives because it gave the best contrast between tissue types. This increased the capability of the software to detect the chosen tissue fibres. The programme determined the detection threshold value because the manual setting of the maximal value proved difficult due to variation in the amount of collagen in various samples.

Slides were also used to count the number of mucosal folds in the Fürstenberg’s rosette for comparison with the number of macroscopically visible folds in the silicone models of the same teats (Vesterinen et al., 2015). This microscopic and macroscopic comparison included all four teats from ten cows.

### 2.2 Statistical analyses

The IBM SPSS Statistics 25.0 software was used for the descriptive statistics and to analyse the correlation (Spearman’s 2-tailed correlation test) between the amounts of the tissue types of the same section. The number of tissue folds seen in the histological sections and silicone models made from the same teats were analysed with the paired t test. \( p \)-values < 0.05 were considered statistically significant.

### 3 RESULTS

The image processing software was able to estimate the amount of elastic fibres (mean = 16.34, SD = 4.12, \( N = 49 \)) and collagen (mean = 22.07, SD = 5.42, \( N = 62 \)) in the teat tissue sections but the estimations varied according to the magnification. No correlation was observed between the amounts of these tissue types of the same section (\( r = -0.14, p = .33 \)). The thickness, shape (straight, curly) and orientation of the elastic fibres varied (Figure 1). The elastic fibres in the rosette folds appeared as dots in the tissue samples cut parallel to the papillary duct and as long fibres in the transverse sections. The elastic fibres were visible as short fibres in tissue sections cut in an angle to the papillary duct. The amount of elastic tissue in the teats of cows with a history or acute case of mastitis infection varied between 10.7 and 23.3 per cent (mean 16.5%). The

**FIGURE 1** The elastic fibres appeared in various shapes depending on their orientation in the tissue section. The arrow points to particularly thin, faintly stained fibres. Aldehyde fuchsin stain. 200 x magnification.
corresponding values in non-infected teats were 9.9-27.6 per cent (mean 16.3%).

The amount of collagen tissue in the teats of cows with a history or acute case of mastitis infection varied between 15.8 and 34.2 per cent (mean 22.7%). Corresponding values in non-infected teats were 9.9-40.4 per cent (mean 21.9%). The statistical significance of the variation in the amount of either elastic fibres or collagen could not be tested due to the low number of infected cows in our samples. The arrangement of muscular fibres was not observed, nor could the amount of muscle tissue be estimated with the method used in our study (no special staining for the muscle tissue).

The Fürstenberg’s rosette was formed by longitudinal folds of connective tissue (Figure 2). In many cases, the primary folds consisted of secondary folds, leaving room for interpretation as to the total number of folds seen in cross-sections. The folds extending deep towards the dermis were considered primary folds. The number of mucosal folds in the Fürstenberg’s rosette in histological cross-sections varied between 5 and 14 (mean 7.84). The number of folds in the same teats did not differ significantly between the histological and silicone model samples (4-18 (mean 7.59; Vesterinen et al., 2015); p = .11).

4 | DISCUSSION

The use of an image processing software proved a promising method for estimating bovine teat tissue types between samples of the same study. However, because magnification affects the ability of the software to detect elastic fibres, it is not a representative method for measuring this tissue type in whole teat. Many tissue sections in this study had artefacts that needed to be excluded by hand from the software to allow the software to read the picture correctly. Also, the colour intensity of various histological samples may vary if they are not made or examined simultaneously (due to fading). As the intensity of tissue section colours is critical for the software to recognize tissue types and their amounts, certain samples in this study had to be excluded entirely because of the variation in colour intensity. For that reason, this method is not a reliable way for quantifying the absolute amount of tissue types.

Similar to the findings of Nickerson and Pankey (1983), the Fürstenberg’s rosette was formed by longitudinal folds of connective tissue. The average number of mucosal folds in the Fürstenberg’s rosette in histological cross-sections was very similar to that found in macroscopic silicone casts of the same teats (Vesterinen et al., 2015) despite separation of the secondary folds from the primary folds being difficult in some cases (see also Nickerson & Pankey, 1983). However, further studies concerning the possible effect of the secondary folds on the proper closing of the papillary duct are warranted. Light microscopy also showed that the folds were arranged longitudinally, as reported by Nickerson and Pankey (1983) (not in spiral fashion, as indicated by Giesecke et al., 1972), with no evidence of the folds interlocking.

Condino et al. (2012) reported degradation of elastic fibres in the lamina propria of the teat cistern and the Fürstenberg's rosette in clinically infected cows. Our microscopic estimation of teats of individual cows indicated differences in the amount of various tissues between teats, especially in the collagen content. However, we could not test this statistically. Besides the health histories of cows, future studies should include infection information of each teat when assessing the possible connection between the teat canal tissue composition and susceptibility to mastitis infections. Also, more information is needed concerning the influence of mastitis type (clinical versus. subclinical), breed, milking method and age on teat tissue composition.

In this study, we only assessed the amount of tissue types within a sample area. However, the thickness or orientation of the fibres may also affect the proper closing of the papillary duct. The orientation of the elastic fibres in this study was similar to those of muscle fibres reported in earlier studies (Foust, 1942; Helmboldt, Jungherr, & Plastridge, 1953) with a net-like spiral arrangement. However, more detailed studies on the amount and arrangement of elastic fibres and muscular tissue are warranted (Ozenc et al., 2020). Also, investigation of the possible presence of oxytocin receptors on teat muscular tissue (Bruckmaier, 2005; Ozenc et al., 2020) would offer more information on the opening mechanisms of the teat.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data are available on request due to confidential/proprietary restrictions.
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