Effects of different teat inserts on wound healing of experimentally incised streak canal in non-lactating cattle

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ABSTRACT. The recurrence of reduced milk flow is a common sequela after placement of teat inserts in the streak canal as a treatment for teat injury. We evaluated the effects of three types of commercial teat inserts on wound healing. Thirty-two normal streak canals of eight cows were incised longitudinally using a teat knife. Then, a wax-bougie, silicone self-retaining cannula, and catgut teat dilator were placed in each cow for 7 days. No insert was placed on the remaining teat as a control. Histopathological examination revealed that granulation polyps with squamous metaplasia developed in the area around the rosette of Furstenberg in most teats in which silicone cannulas and catgut teat dilators were placed, whereas the controls and the teats with wax-bougies healed with less tissue reaction. This study suggests that the placement of irritating inserts, such as a silicone cannula and catgut teat dilator, interfere with wound healing.

KEY WORDS: cattle, silicon, steak canal injury, teat insert, wax-bougie

Traumatic injury of the teat end is caused by the digit or medial dewclaw of the ipsilateral limb of the affected cow or injury from neighboring cows stepping on the teat [19]. Some of these injuries are accompanied by limited outer skin laceration [6, 10], which is referred to as covered streak canal injury [6, 7]. Such injuries are likely to cause mucosal separations in the region of the streak canal or rosette of Furstenberg, resulting in reduced milk flow due to fibrosis of mucosal tissue or obstruction of the region [7, 9, 17].

Treatment procedures employed by veterinarians include a non-surgical approach, involving the placement of a teat dilator into the streak canal in acute cases, and surgical correction with incision or excision of the lesions followed by placement of a teat insert in chronic cases, depending on the site of injury, fibrosis, or obstruction of granulation tissue [9]. The teat insert can widen and stabilize the streak canal until the wound heals [6–9]. However, after either procedure, recurrence of reduced milk flow is common [9]. Such recurrence is due to the relapse of fibrosis or the development of excessive granulation tissue, but the factors related to these problems have not yet been well-investigated. One such factor may be the blind use of surgical instruments through the teat orifice, which can produce iatrogenic lesions, such as extensive, blunt mucosal damage or defects, including those of healthy tissue [4, 6, 9]. The development of excessive granulation tissue that can obstruct the teat is a common result of extensive mucosal damage or defects in the teat cistern [6, 15, 18] and the rosette of Furstenberg [9].

Another possible factor is the placement of teat inserts that act as a foreign body and irritate the mucosa. A prosthesis made for teat inserts placed in the teat cistern can irritate and stimulate granulation tissue development at its contact point, with proximal or distal mucosal lesions [6]. Therefore, we placed different commercially available inserts of different shapes and materials in experimentally incised teats and evaluated their effects on wound healing and the causes of recurrence.

MATERIALS AND METHODS

All procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals and at the clinical practice standard of the Central Research Institute, Nippon Zenyaku Kogyo Co., Ltd (NZ99/47-01C-1). This study was performed on eight non-lactating Holstein cows that had normally shaped teats and teat ends without pathological conditions,
which were confirmed by careful inspection, palpation, and ultrasonography before the experiment.

The three different teat inserts used in this study are shown in Fig. 1. Insert A was a wax-bougie, which is similar in composition to natural teat sebum, which was found to adjust to the shape of the streak canal after placement for 5 days by dissolution (Natural teat insert, Eickemeyer, Tuttingen, Germany) [7]. Insert B (A-tube, Fujihira Kogyo, Tokyo, Japan) was a silicone cannula, which has a bulge at the proximal end for self-retention and a distal end that can be capped between milking. Insert C was a gut string teat dilator made of catgut that was braided in a stiff stick fashion and gradually macerated upon placement into the teat (Teator Stick, Nihon-Yakuhin, Tokyo, Japan). Insert A and B were sterilized products, but there was no information available regarding the microbial status of Insert C. Therefore, Insert C was sterilized using ethylene oxide gas before use.

The cows were sedated with 2% xylazine hydrochloride (Celactal, Bayer, Osaka, Japan) at a dose of 0.1 mg/kg via intravenous injection and placed in dorsal recumbency. After the mammary gland and teats were aseptically prepared, the teats were locally anesthetized by infiltrating 5 ml of 2% lidocaine hydrochloride (Lidoquick, Terumo Co., Tokyo, Japan) at the base of each teat in the form of a ring block and by the insertion of 3 ml into the teat sinus. The streak canal was incised longitudinally with a teat knife (Hug’s teat lancet, Fagor, Sialkot, Pakistan) from the internal streak canal opening (rosette of Furstenberg) to the external opening (teat orifice). Four longitudinal incisions were made at 90-degree intervals. After the incisions were made, intramammary antibiotic ointment (Spectrazol, Tanabe Seiyaku Co., Tokyo, Japan) was infused into each teat cistern through the streak canal.

Thirty-two teats of eight cows were subjected to one of four treatments, with eight teats per treatment group, in a Latin square design. The treatments included 7-day placement of Insert A, B, and C, and no placement (control). All teats were bandaged with elastic adhesive tape (Elastoplasts 5 cm, Eickemeyer, Germany), which was applied as a longitudinal U-shaped layer and then a circular layer.

Histopathological examination

The cows were euthanized 7 days after placement of the teat inserts. The teats were immediately amputated and fixed in 10% neutral-buffered formalin. Tissue samples were collected from four transections of the teat; the teat orifice (distal tip of the streak canal), middle part of the streak canal, rosette of Furstenberg (proximal entry of the streak canal), and distal teat cistern, for histopathological studies. The specimens were embedded in paraffin wax, sectioned at a thickness of 5 µm, and stained with hematoxylin and eosin according to standard procedures. The assessed histopathological characteristics were mucoepithelial necrosis, hemorrhage, leukocytic infiltration, fibrosis, and other findings in the four transections of each teat. These four characteristics were subjectively scored using a scale ranging from 0 to 3 (0 = no lesions present, 1 = lesions present in an area less than one-third of the specimen, 2 = lesions present in an area from one- to two-thirds of the specimen, and 3 = lesions present in an area greater than two-thirds of the specimen). The other findings recorded were histopathological lesions and the number of specimens on which these lesions were observed.

Statistical analysis

The nonparametric Steel-Dwass test (StatLight, version 2.0, Yukms Co., Ltd., Tokyo, Japan) was used to analyze whether the distribution of histological scores differed among the four treatment groups. P-values <0.05 were considered significant.

RESULTS

During the experimental period, clinical mastitis with swelling and redness of the udder were not observed, and none of the cows developed other clinical diseases. Results from control teats and teats in which teat inserts were placed are summarized in Tables 1–5 and illustrated in Fig. 2.

The streak canal incisions healed with less scar formation in most control specimens and teats with Insert A (Fig. 2a). In control specimens, the median histopathological scores of mucoepithelial necrosis, hemorrhage, leukocytic infiltration, and fibrosis at all locations of the teat were low (0 to 1), except for the leukocytic infiltration score at the rosette of Furstenberg. No median histopathological scores of specimens in which Insert A had been placed differed significantly from those of the controls (Tables 1–4).
The median histopathological scores of the four characteristics of the Insert B and C specimens were relatively high, but there were no significant differences at all locations of the teat (Tables 1–4). At the streak canal, the median leukocytic infiltration and fibrosis scores of the Insert C specimens were significantly higher than those of the Insert A specimens (Fig. 2a–b, Tables 3 and 4). The specimens in which Insert B had been placed were found to have more severe leucocytic infiltration and fibrosis than Insert A specimens (Fig. 2a, 2c), although there was no significant difference between them (Tables 3 and 4). At the rosette of Furstenberg, the Insert B and C specimens had significantly higher scores for all pathological characteristics than the Insert A and control specimens (Tables 1–4). Marked infiltration of neutrophils and lymphocytes with fibrosis was detected at the incision sites and extended into the surrounding area, with destruction of the rosette of Furstenberg (Fig. 2d). In most specimens in which Insert B

### Table 1. Median (minimum-maximum) mucoepithelial necrosis score with insert materials at different anatomical areas of the teat

| Location                 | Control     | Insert A    | Insert B    | Insert C    |
|--------------------------|-------------|-------------|-------------|-------------|
| Teat orifice             | 0.5 (0–1)   | 1.0 (0–1)   | 2.0 (1–2)   | 2.0 (1–3)   |
| Streak canal             | 0.5 (0–1)   | 1.0 (0–1)   | 1.0 (1–3)   | 1.5 (1–3)   |
| Rosette of Furstenberg   | 0.0 (0–1)   | 0.0 (0–1)   | 1.0 (0–3)   | 1.5 (0–3)   |
| Distal teat cistern      | 0.0 (0–2)   | 0.0 (0–0)   | 1.0 (0–3)   | 1.0 (1–3)   |

Significant differences (P<0.05) are indicated between different superscript letters.

### Table 2. Median (minimum-maximum) hemorrhage score with insert materials at different anatomical areas of the teat

| Location                 | Control     | Insert A    | Insert B    | Insert C    |
|--------------------------|-------------|-------------|-------------|-------------|
| Teat orifice             | 1.0 (0–2)   | 1.0 (0–2)   | 2.0 (1–2)   | 1.0 (1–2)   |
| Streak canal             | 1.0 (0–1)   | 0.5 (0–2)   | 1.0 (0–2)   | 1.0 (0–2)   |
| Rosette of Furstenberg   | 0.0 (0–0)   | 0.0 (0–0)   | 1.0 (0–3)   | 1.0 (0–2)   |
| Distal teat cistern      | 0.0 (0–1)   | 0.0 (0–0)   | 0.0 (0–2)   | 1.0 (0–2)   |

Significant differences (P<0.05) are indicated between different superscript letters.

### Table 3. Median (minimum-maximum) leukocytic infiltration score with insert materials at different anatomical areas of the teat

| Location                 | Control     | Insert A    | Insert B    | Insert C    |
|--------------------------|-------------|-------------|-------------|-------------|
| Teat orifice             | 1.0 (0–1)   | 1.0 (0–2)   | 2.0 (1–2)   | 2.0 (1–2)   |
| Streak canal             | 0.0 (0–1)   | 0.5 (0–1)   | 1.0 (1–2)   | 2.0 (1–3)   |
| Rosette of Furstenberg   | 0.0 (1–3)   | 0.0 (1–2)   | 2.0 (2–3)   | 2.0 (2–3)   |
| Distal teat cistern      | 0.0 (1–3)   | 0.0 (0–2)   | 2.0 (1–3)   | 2.5 (2–3)   |

Significant differences (P<0.05) are indicated between different superscript letters.

### Table 4. Median (minimum-maximum) fibrosis score with insert materials at different anatomical areas of the teat

| Location                 | Control     | Insert A    | Insert B    | Insert C    |
|--------------------------|-------------|-------------|-------------|-------------|
| Teat orifice             | 0.5 (0–1)   | 1.0 (0–2)   | 2.0 (1–2)   | 1.5 (1–2)   |
| Streak canal             | 0.0 (0–1)   | 0.5 (0–2)   | 1.0 (1–2)   | 2.0 (1–3)   |
| Rosette of Furstenberg   | 0.0 (0–2)   | 0.0 (0–1)   | 2.0 (0–3)   | 2.0 (1–3)   |
| Distal teat cistern      | 0.0 (0–2)   | 0.0 (0–1)   | 2.0 (0–3)   | 2.5 (2–3)   |

Significant differences (P<0.05) are indicated between different superscript letters.

### Table 5. Comparison of the incidence (%) of granulation polyps in different anatomical areas of the teat with commercial inserts

| Location                 | Control     | Insert A    | Insert B    | Insert C    |
|--------------------------|-------------|-------------|-------------|-------------|
| Teat orifice             | 0           | 0           | 0           | 0           |
| Streak canal             | 0           | 0           | 0           | 0           |
| Rosette of Furstenberg   | 0           | 75.0%       | 62.5%       | 87.5%       |
| Distal teat cistern      | 0           | 75.0%       | 62.5%       | 87.5%       |

The median histopathological scores of the four characteristics of the Insert B and C specimens were relatively high, but there were no significant differences at all locations of the teat (Tables 1–4). At the streak canal, the median leukocytic infiltration and fibrosis scores of the Insert C specimens were significantly higher than those of the Insert A specimens (Fig. 2a–b, Tables 3 and 4). The specimens in which Insert B had been placed were found to have more severe leukocytic infiltration and fibrosis than Insert A specimens (Fig. 2a, 2c), although there was no significant difference between them (Tables 3 and 4). At the rosette of Furstenberg, the Insert B and C specimens had significantly higher scores for all pathological characteristics than the Insert A and control specimens (Tables 1–4). Marked infiltration of neutrophils and lymphocytes with fibrosis was detected at the incision sites and extended into the surrounding area, with destruction of the rosette of Furstenberg (Fig. 2d). In most specimens in which Insert B
or C had been placed, granulation polyps developing from incision sites were present at the rosette of Furstenberg and distal teat cistern (Table 5). The polyps continued from the columnar mucosal epithelium of this region and consisted of abundant fibrous tissue and squamous epithelium, which suggested squamous metaplasia (Fig. 2e). The polyps partially occluded the lumen of the teat in severe cases. No polyps were found in the control or Insert A specimens.

**DISCUSSION**

In this experimental study, we attempted to model the clinical situation of the use of different types of teat inserts by veterinarians to treat streak canal injury as a conservative procedure or after streak canal surgery without consideration of the adverse effects [9], which can result in the recurrence of reduced milk flow. This study demonstrated that Insert B and C, following streak canal incision, interfere with wound healing, suggesting that they are irritating inserts. The notable adverse effects were extensive leukocytic infiltration and excessive granulation tissue formation at and around the rosette of Furstenberg. On the other hand, the teats in which Insert A was placed and the control teats (no placement of an insert) healed with less tissue reaction, suggesting that Insert A, composed of natural teat sebum, is a nonirritating insert without adverse effects, as reported previously [7, 8].

The common site showing development of granulation polyps suggested that the area at and around the rosette of Furstenberg...
is a key site for restoring the patency of streak canal injury. This is consistent with most teat stenosis due to injury occurring at the rosette of Furstenberg and distal teat cistern [18]. The extensive inflammatory reactions and development of excessive granulation tissue at the rosette of Furstenberg can be explained through two different mechanisms. First, the rosette of Furstenberg is a sensitive site of the squamocolumnar epithelial junction between the streak canal and teat cistern [1], which acts as part of the protective barrier against microbial invasion and is the location that lymphocytes preferentially accumulate and infiltrate with other inflammatory cells through the bistratified columnar epithelium [6, 12]. Second, the mucosa of the teat cistern, including the proximal site of the rosette of Furstenberg covered by a thin bistratified columnar epithelium, has structures that loosely attach to the underlying tissue, which has a rich blood supply, making it prone to excessive granulation tissue formation. Squamous metaplasia of the bronchial epithelium due to chronic irritation and in a duct of a mammary gland with chronic mastitis in cattle has previously been reported [20]. The squamous metaplasia found with granulation polyps was considered to be a response to irritation and inflammation caused by the irritating inserts.

Even the sharp and fresh incisions at the rosette of Furstenberg were unable to heal properly compared to the controls when the irritating inserts were in place. Surgical correction of the streak canal comprises two procedures; incision of the tight teat sphincter and proximal aspect of the streak canal (the rosette of Furstenberg), and excision of the tissue flap at the rosette of Furstenberg, which interferes with milk flow, similar to valve action [18]. In either procedure, the rosette of Furstenberg is usually involved. Therefore, any surgical correction procedure can be harmful if the inserts are placed following surgery. Although blunt excision of the tissue flap involving the rosette of Furstenberg results in almost 100% recurrence of teat stenosis [6], the placement of irritating teat inserts may be partly responsible for this high recurrence rate.

The irritating Insert B and C may produce cellular responses mechanically or chemically in the surrounding tissue, thereby inducing a tissue response. Insert B, a teat dilator and a cannula, had a distinctive shape, consisting of a constriction of 4.2 mm in diameter and a self-retaining bulge with a maximum diameter of 8.7 mm. The bulge at the rosette of Furstenberg, which partly invaginated into the proximal streak canal, was sufficient to cause mechanical damage, even though it was made of inert silicone. A teat cannula cone 5 mm in diameter was reported to damage the streak canal and rosette of Furstenberg [13], whereas a short and rod-shaped silicone implant prevented adhesion of the streak canal without adverse effects after teat surgery [14].

Insert C, made of catgut, was initially hard, coarse, and long, which may have caused it to damage the streak canal and rosette of Furstenberg until maceration by brushing against structures, causing the tip to pierce the cistern lining when the cow lay down, resulting in the bending of the teats [3, 7, 13, 16]. Insert C may also be a chemical irritant to the surrounding tissue, as the catgut sutures may induce moderate inflammatory reactions in tissue during enzymatic degradation and phagocytosis [2]. Indeed, the placement of Insert C resulted in similar tissue reactions to Insert B, even though it was rod-shaped with a thinner diameter (3 mm) than Insert B.

The retarded healing of the streak canal incisions in the teats in which Insert B and C were placed may be explained by the inoculation of bacteria growing in the teat. Teat cannulas and dilators may impair the defense mechanisms of the teat and promote infection [13]. Teats exhibiting fibrosis and neutrophil and mononuclear cell infiltration with or without mammary gland infection have a grossly normal appearance [5, 11]. We did not examine the somatic cell count or bacterial levels in the milk of the cows, which could have suggested whether an infection of the teats or udders was present, because the experiments were carried out using non-lactating cows. Therefore, it was not possible to assess whether the teats and udders were infected during the experiment.

Couture and Muloin suggested that complete cessation of milking upon placement of a teat insert can improve streak canal injury [6]. The present study cautions that the application of irritant inserts, such as Insert B and C in this study, to injured or surgically managed streak canals often produces granulation polyps at and around the rosette of Furstenberg, which may cause the recurrence of teat stenosis. In conclusion, nonirritating teat inserts, such as Insert A in this study, should be used for treatment associated with the surgical correction of the streak canal or as an initial treatment for streak canal injury.

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