



**Original Study**

# Use of Conjunctival Grafts and Temporary Tube Implants for Treatment of Teat Sinus Mucosal Injuries

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## Abstract

**Objectives-** Using conjunctival graft to replace experimental mucosal defects of teat sinus.

**Study-** Experimental study.

**Animals-** Five healthy, mature, lactating, Jersey cows.

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**Methods-** Conjunctival mucosal grafts with teat cannula were evaluated in the repair of experimental teat sinus mucosal lesions. A 1.5×1 cm wide circumferential strip of mucosa was resected from each teat in five Jersey cows. Each lesion was then treated by one of the three methods: 1. teat cannula (C); 2. Conjunctival mucosal graft and teat cannula (C+G); 3. Only mucosal graft (G). All teats were evaluated 4 weeks after treatment, for patency and luminal diameter by radiography and ultrasonic examination. The cows were euthanized, and teats were collected for histopathologic study.

**Results-** The histopathologic and radiographic results showed that the conjunctival tissue of the teat was incorporated by the teat mucosal tissue. Some acanthosis in conjunctiva was observed, due to the stimulation of the teat cannula.

**Conclusion and Clinical Relevance-** Conjunctival grafts can be used in teat mucosal defects when other therapeutic methods fail to keep the integrity of the teat sinus mucosa.

**Key Words-** Conjunctival graft, Teat sinus, Cow

## Introduction

Obstruction is commonly referred to as stricture or stenosis, with the implication that the lumen narrows as a result of wound contraction<sup>1,2,3,4</sup>. Because of its physiological and anatomical peculiarity, the cow's teat is exposed to more vigorous manipulation and a greater risk of injury than any other structure of the animal body<sup>5,6</sup>. Injuries to the teat can be caused by various factors, including trauma, infection, husbandry practices and environmental factors<sup>5,7</sup>. Regardless of etiology, teat lesions become important because of the economic impact they have on the dairy enterprise. Affected teats often become difficult to milk and milking efficiency declines. In addition, teat lesions are at risk of infection, which may then exacerbate the severity of the lesions or lead to mastitis.<sup>7</sup>

Treatment techniques have been used to provide or restore normal function in partially or completely obstructed teat lumen, including incision or dilation of the site of obstruction with instruments introduced through the teat canal<sup>4</sup>, direct exposure of the lesion via thelotomy and excision of obstructing tissue<sup>8</sup>, insertion of a prosthetic device via the teat canal<sup>4</sup> or via thelotomy<sup>1</sup> to bridge the obstruction site and maintain a patent conduit for milk flow.

The study reported here was designed to evaluate the use of mucosal grafts in combination with teat cannula as a method of preventing obstruction after mucosal rejection without relying on a permanent implant. The study was done based on the hypothesis, cited by Trent et al. (1990),<sup>9</sup> that by improving the rate or quality of mucosal defect coverage, the stimuli for production of obstructing tissue or for wound contraction leading to stricture would be decreased.

## Materials and Methods

### Animals

Five healthy, mature, non gravid jersey cows near the end of their lactation period were used. Cows were screened for systemic and localized intramammary inflammatory disease before the operation, by physical examination and evaluation of cell blood count, and bacteriologic culture of individual mammary gland milk samples. Xylazine hydrochloride (0.08mg/kg, IM), followed by high epidural analgesia was performed for surgery.

### Lesion and Examination Groups

**Lesion production**—Surgical preparation and draping of the teats, eyelid and conjunctivae were performed, under high epidural analgesia. A pair of doyen forceps was inserted at the base of the teats to prevent mammary secretion and hemorrhage and a teat cannula was introduced into the teat cistern. A vertical full-thickness incision extending the length of the teat sinus, but not entering the teat papilla or gland sinus, was made on the medial or lateral surface of each teat. A 1×1.5 cm piece of teat mucosa, at the mid-point of the

teat sinus m, was sharply resected from the teat lumen and following hemostasis, each teat received its reassigned treatment. After treatment all teats were closed in 3 layers, using 4-0 polyglactin 910, in a simple continuous penetrating pattern for the mucosal layer (incorporating submucosal tissue at the level of mucosal resection), 4-0 polyglactin 910 in a simple continuous pattern for the submucosa/muscularis, and 3-0 monofilament nylon in a simple interrupted pattern for the skin.

**Examination groups**—Three treatments were used: conjunctival graft only (G); teat cannula only (C); and teat cannula plus conjunctival graft (G+C).

During preparation of the teat in cows of group G and C+G a second team of surgeons harvested the conjunctival graft from the left upper eyelid. The upper eyelid was rolled dorsally to expose conjunctiva and prepared for surgery. A rectangular area of conjunctiva with a width of at least 3cm was outlined with scalpel. The corner of each conjunctival flap was elevated, and the flap was freed from the underlying conjunctival tissue by a combination of blunt and sharp dissection. After flap removal the conjunctival defect was closed by a 4/0 polyglactin 910 suture. Conjunctival flaps divided into 1.5×1 cm linear strips and placed in gauze moistened with sterile isotonic saline solution until the graft beds were prepared. One strip was placed longitudinally in each G or C+G recipient site. The graft was placed in the teat lumen and sutured by 4-0 polyglactin 910. The teat was then closed as previously described. For cows of group C, the mucosal defect was left open, the teat wall closed as previously described, and a teat cannula inserted in the teat cistern.

In cows of the C+G group, teat lesions were treated as group G followed by insertion of a teat cannula in the cistern.

**Teat cannula removal**—Two weeks after lesion creation, all cows were again tranquilized and restrained on right lateral recumbency over the surgical table. Each teat was evaluated for gross patency, and secretions were collected from patent teats for culture and antimicrobial susceptibility evaluation after milk sample collection, sutures and teat cannula was removed.

#### *Evaluation*

Gross patency was evaluated by manual expression of secretion from the teat.

**Radiographic evaluation**—Before and 4-weeks after surgery radiographic evaluation was performed with the cows restrained in right lateral recumbency by double contrast radiography with contrast air pressure previously reported by molaei et al. (2002).

**Ultrasonographic evaluation**—Ultrasonographic studies of all teats were done before surgery and then 2 and 4 weeks after surgery. All ultrasonographic imaging of the teats were carried out on cows in the standing position, with an ultrasound scanner (sonoace 600v; Kretz-Technik) fitted with a 6.5-8.5 MHZ linear transducer.

**Histopathological evaluation**—Cows were slaughtered within 48 hours of the final radiographic and ultrasonographic examination, and teats were removed immediately after slaughter, and fixed in 10% neutral buffered formalin for histopathological evaluation. The teats were then sectioned into 5-mm- thick, transverse slabs from teat tip to base. Four slabs were selected from each teat for sectioning: 1 slab distal to the lesion; 2 central on the lesion; and 1 proximal to the lesion. All sections were evaluated; using hematoxylin and eosin staining method, and studied for mucosal repair at the site of lesion and the character of the mucosa.

#### *Statistical analysis*

For comparison of changes in luminal diameter during the time in the two groups, two-way analysis of variance and the level of significance of 0.05 were used. Duncan test was used for comparing the means. SPSS software was used for statistical analysis.

## **Results**

**Gross patency**—All teats in group C and G were obstructed two weeks after the surgery. At this time, all teats in group C+G were patent but 2 weeks later 9 of 10 teats were patent.

**Radiological results**—As shown in tables 1 all teats in groups G + C were patent (fig 1).

**Ultrasonographic findings**—Two weeks after surgery teats in the G group were occluded, while teats in the C group were patent and tissue under the skin were more echogenic than normal at the site of mucosal injury, and there was no sharp contrast between them and the teat cavity. At this time, teats in the G+C group, were patent and mucosal layer in the teat cistern at the site of injury, were more echogenic than the normal teat cistern.

Four weeks after surgery, all teats in the G and C group, were obstructed. At this time, 9 of 10 teats in the C+G group, were patent, despite patency of teat cistern, tissue under the skin were more ecogenic than normal and there was severe luminal narrowing in teat cistern at the site of mucosal injury. Additionally, the secretion inside the cistern showed increased echogenicity, due to the presence of hyperechoic flakes (fig 2).

**Histopathological findings**—Mucosal coverage, as determined by the area of the lesion surface covered by mucosal tissue, varied with the treatment used. Three out of five teats in group C had some mucosal lesions, consisting of a few focal islands of epithelial cells. Two out of five teats in group G had partial mucosal coverage, although none were completely covered. All teats in group C+G had mucosal tissue at the level of the lesion, and nine patent teats had >75% mucosal coverage.

Granulation tissue and mucosal alteration were the most consistent sources of luminal compromise in teats of this study, the distribution of each varying with the treatment group. Submucosal fibrosis was present to a lesser extent in some teats; however, its contribution to luminal compromise was difficult to assessed and was not quantified.

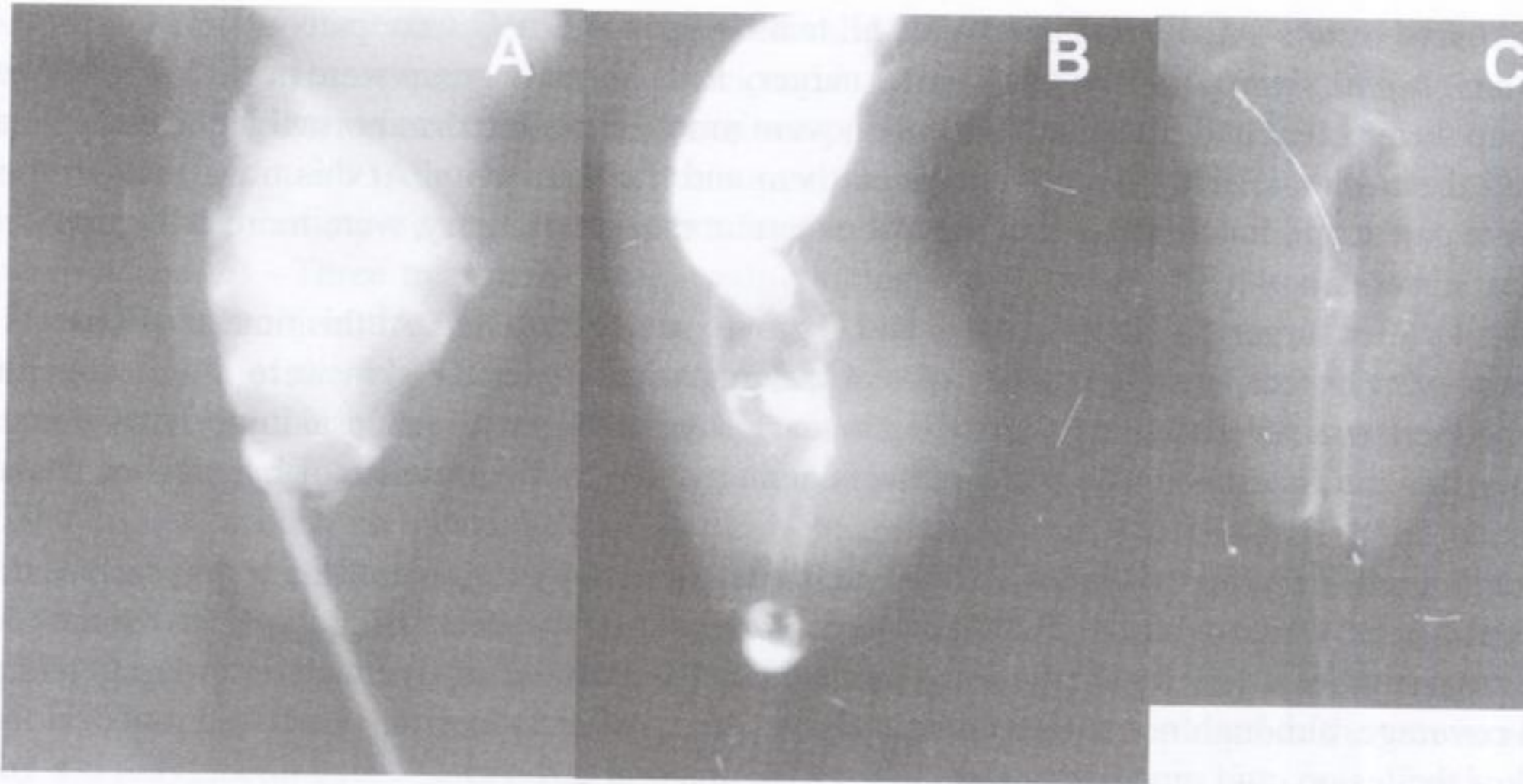
Mucosal alteration was only identified in teats in which grafts were used (G, G+C). Changes included squamous metaplasia, hyperkeratosis, and parakeratosis. The magnitude of these changes as a group was greater in the G-treated teats, as reflected in the lower patency rate for group G. Parakeratosis was the most common mucosal reaction in G-treated teats, but was also observed infrequently in cows of the C+G- and G-treated teats, whereas squamous metaplasia was seen in two G-treated teats but was not identified in C+G treated teats (fig3). Conversely, in C-treated teats, lesional mucosa, which contributed minimally to luminal compromise, was seldom observed.

Granulation tissue proliferating into the lumen was seen in cows of all treatment groups, but represented the sole source of obstruction in G-treated teats.

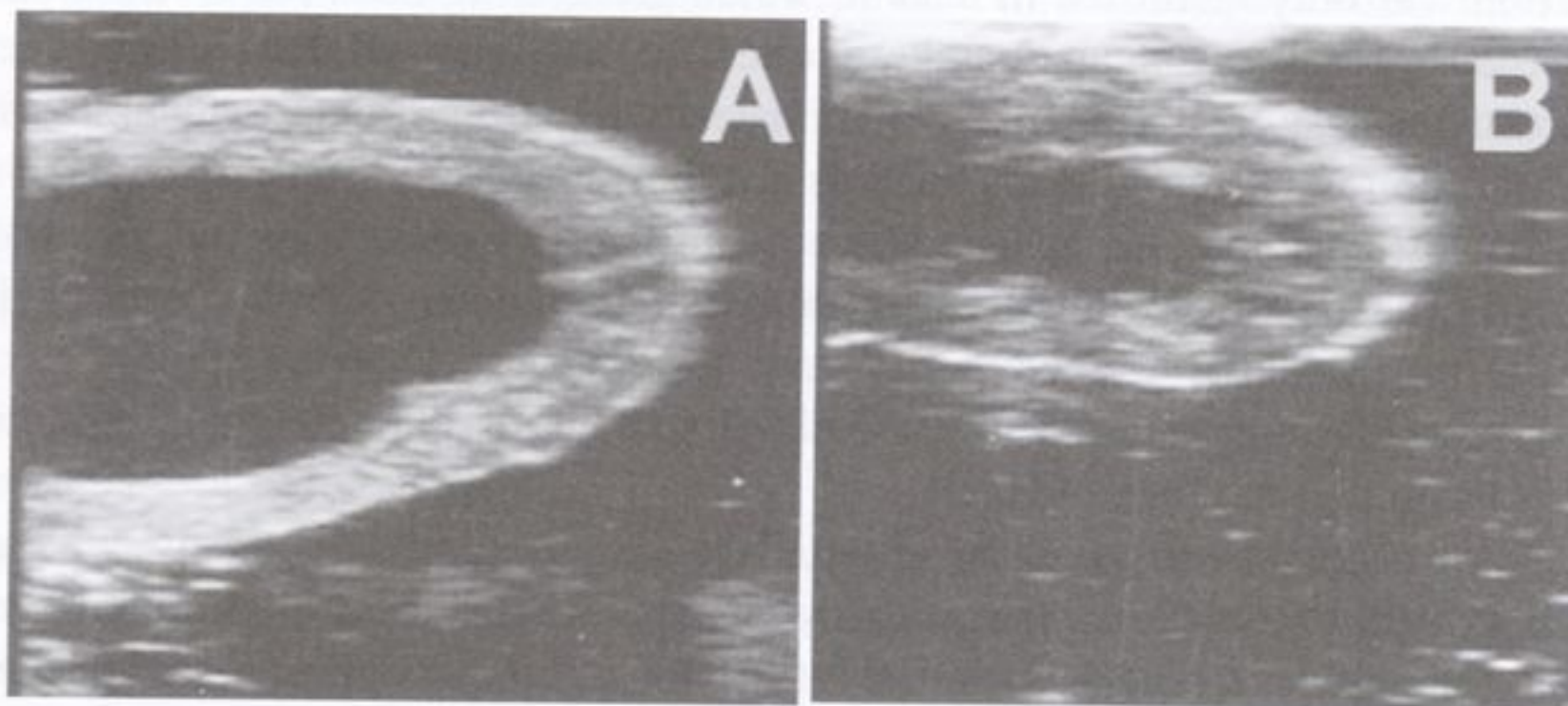
The main cause of cisternal narrowing in group C+G was submucosal fibrosis, which resulted in a moderate narrowing. Mild to moderate inflammatory changes were seen at the lesional level in all teats. The most common changes was a mild submucosal infiltration of lymphocyte along with a few neutrophils, localized suture granulomas were identified in 75% of all teats, with no significant association with treatment group, preoperative teat diameter, or teat position (fig 3).

**Table 1:** Means of radiographic midsinus diameter

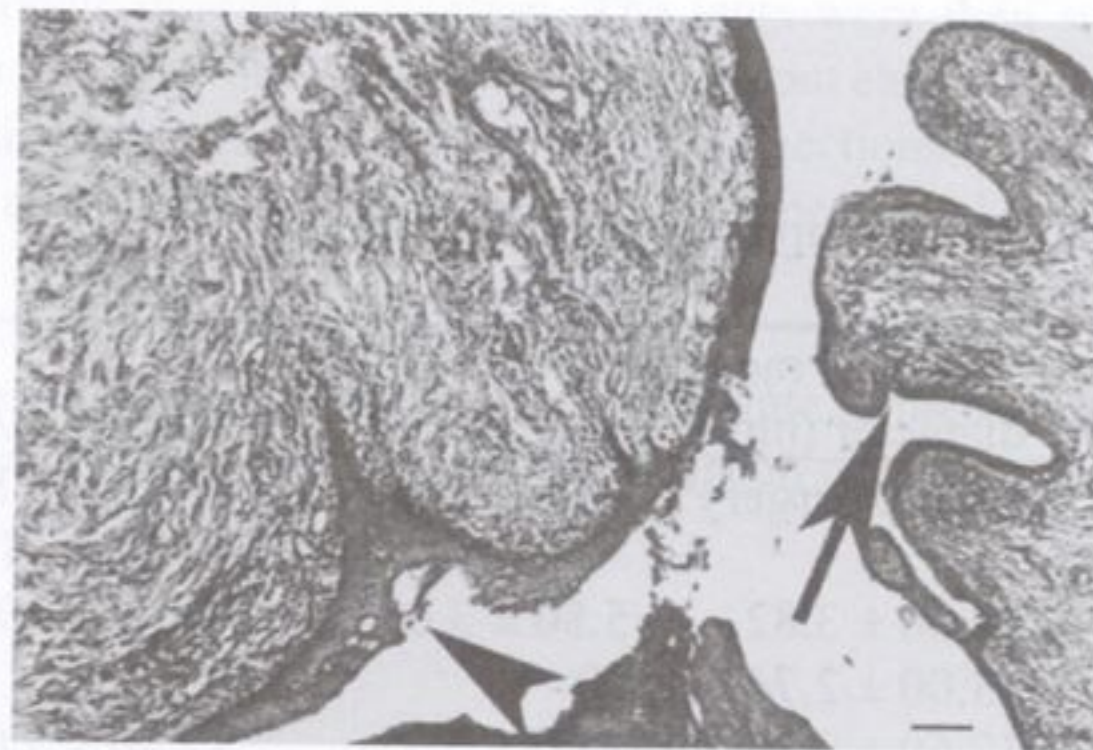
group	N	Means of radiographic midsinus diameter (mm) ± S.D.		Means of After surgery ÷ Before surgery ×100
		Before surgery	After surgery	
G+C	10	12.80 ± 3.82	5.80 ± 3.08	45.60 ± 20.58
G	5	13.00 ± 2.74	00	00
C	5	12.60 ± 2.30	1.60 ± 0.55	12.40 ± 3.05
total	20	12.80 ± 3.20	3.30 ± 3.38	25.90 ± 25.12



**Fig 1.** Double contrast radiography with air pressure in teat cistern: (A) before surgery, (B) after surgery with narrowing in teat cistern( group C+G), (C) after surgery with obstruction in teat cistern( group G)



**Fig 2.** Sagittal scan of the teat: (A) before surgery, (B) after surgery in group C+G



**Fig 3.** Teat sinus mucosal (arrow) treated with conjunctival graft (arrow head). 400- H&E stain; bar = 1cm.

## Discussion

The use of grafts to enhance the rate and quality of epithelial coverage of a wound defect is well documented in tissues other than the teat sinus in various species.<sup>18-21</sup> Although donor mucosa from the mammary system is not readily accessible in cows, other autogenous sources of mucosa are. Oral and vaginal mucosa were tested as possible graft sources in preliminary studies by the investigators.<sup>5,9</sup> The usage of oral mucosa has been reported in studies by Bristol (1989) and Trent et al (1990),<sup>9,10</sup> and the usage of vaginal mucosa has been reported in a study by Molaei et al (2002). According to Trent et al (1990), oral mucosa was found to be preferable, based on relative ease of collection, trimming, and shaping to the desired form and subjectively judged lower associated with mastitis. However, despite careful debridement of submucosal tissue, the oral graft was subjectively judged to be three to four times thicker than the resected mammary mucosa. Non-keratinized transitional epithelium of conjunctiva is more suitable than keratinized stratified squamous epithelium of oral mucosa for treatment of teat mucosal injury.<sup>11,16</sup>

Obstruction of the teat following teat mucosal injury can be caused by granulation tissue formation,<sup>1,3,12,13</sup> fibrous tissue,<sup>8,12-15</sup> and epithelium (mucosa) or epithelial products, such as keratin.<sup>13</sup> In the study by Trent et al (1990),<sup>9</sup> proliferative granulation tissue, epithelium and keratin contributed to luminal narrowing, whereas this study and the study by Molaei et al (2002) showed that moderate submucosal fibrosis in group C+G was considered to be the cause of luminal narrowing. Proliferative granulation tissue and keratin were lower than previous study.<sup>5</sup> Vestibular mucosa is composed of stratified squamous epithelium and thicker than the teat cistern mucosa; while conjunctiva is composed of non-keratinized transitional squamous epithelium, so that unlike the oral and vestibular mucosal grafts, the role of keratin and epithelium in luminal narrowing is not very distinct.<sup>5,16</sup>

Although the graft bed resulting from a teat sinus lesion provides a good vascular supply for graft intake, the luminal surface of the teat sinus is a concave and convoluted surface that presents an unfavorable contour for good graft contact.<sup>9</sup> Silastic tubes,<sup>10</sup> polytetrafluoroethylene tubes,<sup>17</sup> and teat cannula,<sup>5</sup> have been used in the teat sinus to improve graft-to-graft bed contact and prevent adhesion formation between the sinus walls.<sup>9,17</sup> In this study, like previous study, a disposable teat cannula was used for the experiment. The teat cannula is easy to insert and remove and does not need suturing, which can induce additional foreign body reaction in the region. Besides, permanent usage of silastic tubes can decrease milk production and increase prevalence of mastitis.<sup>9,10,18</sup> The usage of tubes was successful in only 50% of the cases.<sup>18</sup> In this study, the teat cannula was left in place for 2 weeks, because revascularization, which starts 24h after grafting, is completed during 2 weeks.<sup>19</sup> The presence of a cannula in combination with grafts resulted in an obvious improvement in patency at 4 weeks after treatment over treatment with graft only. It can be attributed to improvements in graft-to-graft bed contact and graft stability, resulting in decreased stimulation of exuberant granulation tissue and epithelium although the difference in luminal patency between C+G and C treatment was equally marked. The results of present study was in agreement with the study of Trent et al (1990) which showed the marked difference between C+G treatment and the use of G or C treatment alone, suggested that the temporary use of a teat cannula can improve the quality of healing and subsequent luminal patency in teat sinus mucosal lesions treated with grafts.<sup>9</sup> In addition, the results of this study showed, when cannula used in combination with conjunctival grafts, cannula removal may be possible without compromising patency if appropriate timing for removal can be established.

The results of this study show that using conjunctival mucosal grafts with temporary insertion of a disposable teat cannula can be considered as a method of treating teat mucosal injuries. Harvesting the graft from conjunctiva has some advantages over the usage of vestibular and oral mucosa. After oral mucosal resection, the food intake of the animal declines and oral and vestibular wounds are susceptible to more complications. Also, the thickness of conjunctiva is less than oral and vestibular mucosa, and composed of non-keratinized transitional epithelium, which is superior to the oral and vestibular mucosa, a keratinized epithelium. But before clinical recommendation of the technique, evaluation of the technique in real clinical cases should be performed.

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## استفاده از پیوند مخاط ملتحمه همراه با روان شیر

### برای درمان ضایعات مخاطی سینوس سرپستانک

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**هدف:** روش های درمانی مورد استفاده در ترمیم ضایعات مخاطی سینوس سرپستانک دارای اثرات متفاوتی می باشند. ضایعات مخاطی سرپستانک منجر به ورم پستان و جلوگیری از خروج شیر می شود. هر چند قرار دادن لوله های سنتزی در سینوس سرپستانک موجب باز ماندن مجرا تا زمان وجود لوله می شود، اما خود موجب ایجاد ورم پستان مزمن و انسداد در زمان خروج آن می شود. هدف از این مطالعه پیشنهاد روشی برای درمان ضایعات مخاطی سرپستانک می باشد.

**طرح:** استفاده از مطالعات تجربی

**حیوانات:** پنج راس گاو جرسی سالم و بالغ و شیروار

**روش کار:** یک قطعه از مخاط سینوس سرپستانک به ابعاد  $1 \times \frac{1}{5}$  سانتیمتر از تمام سرپستانکهای حیوانات مورد مطالعه جدا گردید. هر یک از سرپستانکهای ضایعه دیده با یکی از روش های زیر مورد درمان قرار گرفت: ۱- روان شیر، ۲- پیوند مخاط ملتحمه همراه با روان شیر و ۳- پیوند مخاطی به تنهایی. تمام سرپستانکها دو هفته بعد از درمان برای بررسی وضعیت مجرا مورد بررسی قرار گرفته و روان شیرها خارج گردیدند. چهار هفته بعد از درمان تمام سرپستانکها برای بررسی وضعیت باز بودن مجرا و اندازه گیری مجرا با استفاده از رادیوگرافی و اولتراسونوگرافی مورد مطالعه قرار گرفتند. آنگاه گاوهای مورد مطالعه به روش انسانی معدوم شده و سرپستانکهای آنها برای مطالعات آسیب شناسی جدا گردید.

**نتیجه گیری:** این مطالعه نشان داد که استفاده از پیوند مخاط ملتحمه همراه با روان شیر می تواند به خوبی در درمان ضایعات سینوس سرپستانک مورد استفاده قرار گیرد.

**کلید واژه ها:** پیوند مخاطی ملتحمه، روان شیر، سینوس سرپستانک، گاو