THE INFLUENCE OF A TOPICAL HAEMOSTIC AGENT ON TISSUE HEALING IN THE NASAL MUCOSA OF SHEEP FOLLOWING ENDOSCOPIC SINUS SURGERY

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THESIS ABSTRACT

The present study was performed to evaluate the effects of a novel topical haemostatic agent on bleeding and on post operative complications in endoscopic sinus surgery. Complications of this procedure includes post operative bleeding, scarring and adhesion formation.

The aims of this thesis were firstly to establish the presence of eosinophilic chronic sinusitis in sheep and secondly to assess the effectiveness of AMICAR (epsilon amino caproic acid) on intra-operative bleeding and post operative healing.

All sheep underwent endoscopic middle turbinectomy as a standardisation procedure under a general anaesthetic. Full thickness mucosal injuries were created on the lateral nasal wall, ethmoturbinal and the maxillary ostium on both sides. The topical haemostatic agent (AMICAR, epsilon amino caproic acid) was sprayed onto a randomly selected side with or without mucoadhesive methyl cellulose (5 sheep in each group). The control side received a spray of saline of equal volume. The degree of bleeding in the surgical field was graded using a visual analogue scale until total haemostasis was achieved. The grades at 2 minute intervals as well as the time to achieve total haemostasis were recorded. Biopsy specimens were obtained from the lateral nasal wall at day 28, 56, 84 and 112 in a rotating manner. These biopsies were then processed for light microscopy and electron microscopy.

The study was performed on sheep with established chronic rhinosinusitis. These sheep were shown to be infested with Oestrus ovis larvae that induces an eosinophilic sinusitis, both by visualising the larvae and by histological sections of the epithelium and secreted mucous containing eosinophils. A minimum baseline level of 2 eosinophils per high power field was established as the criterion to confirm tissue inflammation and chronic eosinophilic sinusitis. This finding will allow future researchers to confirm the presence of Oestrus ovis infestation and related inflammation.

The topical haemostatic agent used in this study, AMICAR, a lysine analogue showed a significantly reduced grade of surgical field bleeding when used on the lateral nasal wall. In addition there was a non significant trend towards a shorter time to achieve total haemostasis when AMICAR was used. However the use of a mucoadhesive, methyl cellulose did not improve the rate of haemostasis. In the post operative period, the use of AMICAR did not affect the rate of adhesion formation.

However, eosinophilia of the epithelium did not correlate with the severity of bleeding. The total time required to achieve total haemostasis was not affected by AMICAR, either with or without methyl cellulose. No relationship was found between the treatment received and the incidence of adhesion formation. There was no significant difference in the rate of epithelialisation and the treatment received at any of the time points studied. The height of the epithelium was not affected by the treatment. However the percentage area of epithelium covered by mature cilia was significantly higher in the normal saline treated side compared to AMICAR treated side at day 84. But by day 112 the rates of reciliation appear to be similar.

Conclusion

In conclusion AMICAR may be considered for use either during or after sinus surgery to reduce bleeding without any detrimental effects on tissue healing or on the development of adhesions.

Thesis Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available in all forms of media, now or hereafter known.

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INTRODUCTION

1. Chronic sinusitis

1.1 Introduction

Sinusitis is defined as an inflammatory condition of the paranasal sinuses. The prevalence of chronic rhinosinusitis in the general population can be as high has 18% (Jones 1998) which results in a significant social and economic burden on society.

1.2 Pathological Course

Chronic rhinosinusitis is initiated by the obstruction of the osteomeatal complex, which leads to hypoxia, anaerobic metabolism and an acidic environment. With obstruction of the osito-meatal complex, a negative pressure develops within the sinus. The sinus continues to produce mucous which stagnates in the sinus providing an ideal medium for bacterial colonization. As the negative pressure continues secretions and bacteria may be drawn into the sinus cavity with colonization and pus production (Parson and Wald 1996). This acidity not only promotes bacterial colonisation but also inhibits local immune mechanisms (Westrin *et al.*, 1990) which manifests as signs and symptoms of infection and associated inflammation. Often, the initiating factor is a viral infection which can range from mild (coronavirus, rhinovirus) to severe (influenza A, respiratory syncytial virus). The secondary pathogens range from aggressive (pneumococcus group A streptococcus) to minimally invasive species (Morexella, nontypable *Haemophilus influenzae*) and with the chronicity of disease, the pathogens become polymicrobial (Poole 1997).

Allergic fungal sinusitis (AFS) has been categorised as a separate entity (Schubert 2004). Fungal elements can be isolated from the patients' mucus, and a high level of fungal specific IgE and a high level of total IgE are found in the serum (Collins et al., 2004). In non-allergic fungal sinusitis, fungal elements are isolated from mucus but total IgE is not elevated. Allergic fungal sinusitis-like sinusitis (AFS-like sinusitis) is another entity that has been recognised more recently. Host characteristics play an important role in the severity of the disease. Immune compromise, other co-morbidities such as diabetes can increase the severity of sinusitis. Factors such as

immunodeficiency, atopy, mucosal and anatomical abnormalities have an impact on host susceptibility.

Characteristically the mucosa exhibits oedema, desquamation of ciliated pseudostratified columnar epithelium, squamous metaplasia, fibrosis, goblet cell hyperplasia, subepithelial thickening. Polyposis and the disruption of the mucociliary transport mechanism are considered to be end stage signs of severe chronic rhinosinusitis (Gerstner *et al.,* 2004). Polyp formation is a localised mediator dependent reaction of the laminar propria (Appenroth *et al.*, 1998). Ciliary abnormalities include compound cilia, short cilia, abnormal tubules and blebs (Toskala *et al.,* 1995).

1.3 Local Inflammatory signs

Nasal allergy has been established as a risk factor for chronic inflammatory rhinosinusitis (Bertrand *et al.,* 1997). Atopic or allergic patients show a significantly high number of eosinophils in the nasal mucosa (Wang *et al.,* 1994) whereas patients with vasomotor rhinitis showed no significant increase in eosinophils or mast cells compared to normal controls (Blom *et al.,* 1995). Eosinophils migrate into the mucosa and are activated within the nasal mucosa upon a nasal allergen challenge (Wang *et al.,* 1994; Kawabori *et al.,* 1994). The driving force for eosinophilic migration remains unknown. Epithelial cells, fibroblasts and mast cells have the capacity to produce GM-CSF (granulocyte/macrophage – colony stimulating factor), IL-6 and IL-8 (Marini *et al.,* 19992). IL-3, IL-5 and GM-CSF have all been identified as survival factors found within polyps (Ohno *et al.,* 1991). Therefore aberrations within the epithelial tissue or initial inflammation, via cytokine production can lead to migration of eosinophils to the mucosa.

Eosinophilic infiltration of the oedematous polypoid tissue is regarded as a pathological hallmark of chronic sinusitis (Gerstner *et al.,* 2004), irrespective of the presence or absence of atopy (Kaliner *et al.,* 1997). Activated eosinophils release eosinophilic cationic protein (ECP) into the tissue or to the circulation (Wang *et al.,* 1994). In atopic individuals, ECP concentration in nasal secretions was found to increase only after a nasal allergen challenge, reaching a peak level 24 hours later

(Wang *et al.,* 1994). Feger *et al* (1997) found that serial allergic mucin ECP levels appear to correspond with disease severity in some AFS patients while there was no significant difference in the ECP levels between atopic patients with nasal polyposis, AFS patients and allergic rhinitis patients. Appenroth *et al* (1998) reports a significant decrease in the number of active eosinophils and serum ECP levels in patients after treatment with systemic and local corticosteroids. These studies suggest that the number of active eosinophils is a useful marker of the extent of inflammation. Eosinophil number and ECP levels can be used to support the diagnosis of AFS and to monitor response to therapy.

Furthermore, secretions from chronically inflamed sinuses possess proteolytic activity of granulocyte origin (Engquist *et al.,* 1983). The enzymes include proteases released by neutrophils, non-specific collagenase and chymotrypsin-like cationic protein (CCP). These enzymes can interfere with the healing mucosa by proteolytic breakdown of epithelium and subepithelial connective tissue.

1.4 Systemic Inflammatory Signs

Systemic inflammatory symptoms and signs are also manifested. High levels of serum interleukins have long been associated with inflammation. In addition, atopic individuals with sinusitis have a higher titre of serum IgE. ECP, released by activated eosinophils is regarded as a measure of the degree of allergic illness (Appenroth *et al.,* 1998). Measurement of the systemic inflammatory markers may allow therapy to be modulated according to the severity of disease. Systemic steroids may be required if systemic inflammation is manifest.

1.5 Medical Management

Medical therapy is the mainstay of treatment for chronic sinusitis. Topical and systemic decongestants have been used as an adjunct in acute sinusitis. These contain vasoconstrictor agents that act on α-adrenoceptors (Kaliner *et al.,* 1997). Topical or local corticosteroids are used to treat chronic sinusitis. They reduce mucosal swelling and facilitate drainage of sinuses, reduce tissue eosinophilia by causing apoptosis of eosinophils (Saunders *et al.,* 1999) and thereby reduce inflammation (Kaliner *et al.,*

1997). Appenroth (1999) argues that since eosinophilic activation and production of ECP are largely responsible for the local inflammatory reaction and possibly for polyposis, the treatment with local and/or systemic steroids should inhibit polyp formation. A topical steroid, fluticasone was shown to reduce the expression of IL-4, IL-5 and IL-13 in the nasal mucosa thus reducing the inflammation (Hamilos *et al.,* 1999; Kondo *et al.,* 1999). In addition hypertonic saline applied topically causes mucolysis and improves ciliary function.

Antibiotics are not recommended for the treatment of acute sinusitis due to its self limiting course and its viral aetiology. Antibiotics are prescribed for patients with acute or chronic bacterial sinusitis when organisms have been identified. Antibiotics such as beta lactams have been used for decades for *Streptococcus pneumoniae, Haemophilus influenzae* and *Morexella catarrhalis.* With chronicity the bacterial flora becomes polymicrobial, thus oral anaerobes, microaerophilic streptococci, staphylococci and Gram negative enteric flora are commonly found (Brook 1996, Kaliner *et al.,* 1997). In this situation, a broad spectrum antibiotic is usually called for. With the emergence of beta lactamase producing bacteria, beta lactams combined with clavulenic acid has become a popular therapy. Currently, for acute bacterial sinusitis, first line therapy includes amoxicillin, doxycycline, trimethoprim-sulfamethoxazole and erythromycin-sulfamethoxazole (Poole 1997). Furthermore topical use of antibiotics in the form of nasal irrigation has been advocated (Cullen *et al.,* 2000) but aerosolized, nebulized or sustained – release polymers of antibiotics are yet to be tested and still remain the subject of ongoing research.

In addition, allergic fungal sinusitis can be treated with desensitization immunotherapy (Cullen *et al.,* 2000) and systemic steroids. Systemic and topical steroids are used to suppress the inflammation and may restore the mucosa to its normal state (Kaliner *et al.,* 1997). The role of leukotriene receptor antagonists remains to be defined and may be beneficial in patients with aspirin sensitivity and allergic rhinitis (Cullen *et al.,* 2000).

1.6 Surgical Management

Endoscopic Sinus Surgery (ESS) has become the standard technique for the management of chronic rhinosinusitis, after failed medical therapy. Lynch (1921) claimed a 100 per cent cure rate with external fronto-ethmoidectomy in his series of patients (Lynch 1921). However, intranasal approaches were also advocated for approaching the frontal sinus (Mosher 1912). He described in detail the anatomy of the frontal sinus and the intranasal approach to the frontal ostium using a headlight. However an external approach to the ethmoid sinus was advocated due to the important anatomical relations. The precursor to the present nasal endoscope was used by Hirschman in 1903. He used a 4.0mm diameter endoscope to examine the middle meatus and the maxillary antrum. In the 1950s Nehls used improved endoscopes with greater resolution, larger field of vision and accurate colour (Kennedy 1985). Over the following 25 years endoscopic examination of the nasal cavities and the sinus ostia and their use for diagnosis expanded. Stammberger and Kennedy in the mid 1980s further developed the concept of endoscopy in the upper airway and introduced endoscopic surgery with an entirely intranasal approach (Kennedy 1985).

Over the last 20 years the use of the rigid intranasal endoscope has had a profound impact on not only our knowledge of the anatomy and physiology but on the surgical procedure. This instrument provides illumination, views of areas previously unable to monitor and the reference points for surgery (Massegur *et al.,* 1995). The phrase *Functional Endoscopic Sinus Surgery (FESS)* was first coined by Kennedy (1985) to emphasise the potential for re-establishing sinus drainage and mucosal recovery ie, the function of the sinuses.

In the early stages of FESS mucosa was generally stripped from the underlying bone without suction (Kennedy and Brent 1997). Further advances saw the introduction of suction forceps, through cutting forceps, fiberoptic delivery of lasers, microdebriders that can effectively cut tissue and allow the use of irrigated diamond and cutting burs in the nose and sinuses.

The most significant advance in the ESS technique has been the recognition of the importance of mucosal preservation (Kennedy and Brent 1997). Therefore the objective of ESS is to restore normal sinus function and preserve normal intranasal mucosa and mucociliary clearance (Kuhn and Citardi 1997).

1.7 Long term outcomes of endoscopic sinus surgery

Although the majority of patients in follow up studies report improvement in their symptoms (Kennedy and Brent 1997) studies of objective outcomes however, report a significantly high rate of recurrent disease. In 165 patient evaluated, 52% who had polypoid disease reported a subjectively satisfactory recovery following surgery, but still demonstrated endoscopic evidence of persistent or recurrent disease (Vleming and De Vries 1991). In another series of 221 patients 45 per cent showed some evidence of residual disease (Kennedy 1992).

When mucosa is stripped, healing is slow and scarring is often the end result. Moriyama (1996) demonstrated that regenerated mucosa does not develop normal ciliary density. Furthermore new bony growth may occur in these areas and provide a nidus for infection (Kennedy and Brent 1997). This phenomenon is commonly seen in the frontal recess, leading to a complete stenosis and chronic infection. The region of the frontal sinus and recess is narrow and mucosal damage may lead to recurrent disease. The complete stenosis of the frontal recess with mucocele formation is recognised as a risk of endoscopic sinus surgery. Resection of the middle turbinate performed to gain access, may result in frontal recess stenosis and subsequent frontal sinusitis (Stammberger 1994). Partial middle turbinectomy may result in the remnant lateralising and adhering to the medial orbital wall. (Kennedy and Brent 1997).

The most important factors in achieving optimal functional results following ESS are minimising bleeding, scar prevention by minimal mucosal injury, avoidance of middle meatal collapse by the use of stents or packs, minimisation of apposing raw surfaces that leads to adhesion formation. Mucous membrane preservation reduces scar formation by preventing granular tissue formation. Post operative fibrin clot debridement may help prevent scar formation as these clots may become organised into fibrous scars. Another factor that contributes to stenosis and obstruction of

osteomeatal complex is neo-osteogenesis which is usually stimulated by denuded bone or chronic infection (Kuhn and Citardi 1997). Post operative infections and oseteitis are also known complications that lead to recurrence of sinusitis.

2.1 The sheep model of FESS

The sheep model has extensively been used to assess healing of the nasal mucosa not only following basic endoscopic procedures but following the use of various packing material. Sheep nasal sinus surgical model has been chosen due to the gross anatomical (Gardiner *et al.,* 1996) and histological resemblance to human nasal sinuses (Illum 1996). Gardiner et al identified similarities between sheep and human nasal cavity, the orientation of the nasal sinuses and the turbinates (Gardiner *et al.*, 1996). Figure 1 is a coronal CT scan of a sheep head showing the orientation of turbinates.

The sheep model for endoscopic sinus surgery was standardised by Shaw *et al.,* (2001a) defining the method of middle turbinectomy for endoscopic access. In this model the effect of middle turbinectomy was assessed using the rate of ciliation and their function in the regenerating epithelium as end points. The mucociliary transport mechanism was evaluated by measuring the rate of transport of carbon particles over regenerated epithelium. They showed that total middle turbinectomy did not significantly alter either the mucociliary clearance rate or the histology of the mucosa on the lateral nasal wall. The regeneration of mucosa after a partial thickness mucosal removal was shown to be rapid and more complete with restoration of ciliary function than a full thickness removal of mucosa (Shaw *et al.,* 2001b). Thus a full thickness injury can compromise mucociliary transport for a considerable period of time, causing mucus stasis, thus predisposing to bacterial infection.

In addition to the study of the temporal pattern of healing of the epithelium, the sheep model has been valuable in assessing the effects of various nasal packs following surgery. The use of ribbon gauze packing against an intact epithelium was shown to cause a significant loss of nasal mucosa (Shaw *et al.,* 2000). Of note, the use of dissolvable hyaluronic acid-based packs in the sheep nasal cavity following

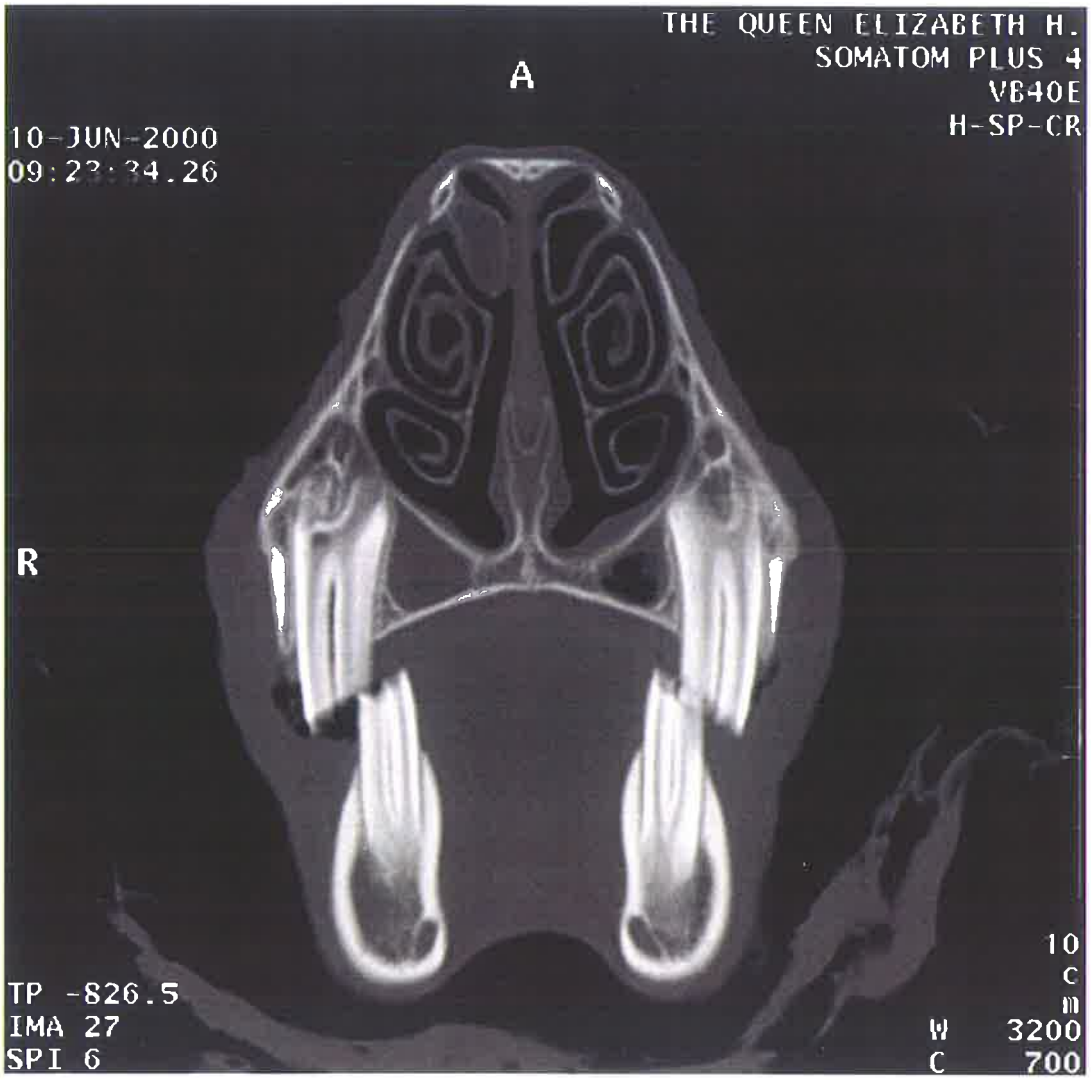


Figure 1: Coronal view of the sheep skull

full thickness mucosal injury significantly improve re-epithelialisation compared to unpacked nasal cavities (McIntosh *et al.*, 2002).

2.2 Sheep model of chronic sinusitis

The sheep is an ideal model for chronic sinusitis because a natural ectoparasite, *Oestrus ovis* induces an eosinophilic sinusitis within the nasal mucosa. Infected animals produce thick and yellowish mucus and show signs of congested nasal passages. This results in snorting and sneezing. Severe infection can interfere with feeding which leads to emaciation. *O ovis* is a dipteran fly that lays its young (L1 larvae) within sheep nostrils. Experimental infestation of lambs with L1 larvae has demonstrated a rise in systemic IgG detectable by ELISA fifteen days after the larvae were deposited (Frugere *et al.,* 2000). The larvae migrate within the nasal cavities and the sinuses and moult toL2 and L3 larval stages. The L3 stage produces an excretory secretory product (ESP) in its saliva that has been shown to be immunogenic (Innocenti *et al.,* 1995) and highly proteolytic (Frugere *et al.,* 2000). The resultant immune reaction is cell mediated as well as humoral. However, serine-proteases produced by the larval digestive tract were shown to be poorly recognised by host antibodies (Tabouret *et al.,* 2003a). When artificially infested with the larvae of the parasite, the local immune reaction seen is well correlated with the larval burden (Nguyen *et al.,* 1999).

Infestation induces a local recruitment of T and B lymphocytes, macrophages and granulocytes (eosinophils, mast cells and globular leucocytes). The leucocyte activation is found to be more prominent in the ethmoid and sinus mucosa where the second and third instar larvae develop (Tabouret *et al.*, 2003b). Nutrition and weight gain is of importance to L3 larvae because they leave the host to pupate on the ground. L3 larvae secrete ESP which is proteolytic and highly antigenic. ESP is used to digest protein fluids such as albumin and mucin (Fugere *et al.,* 2000). The number of mast cells was doubled and the number of eosinophils was up to 58 times higher in the sinus mucosa of infested animals compared to that of control animals (Nguyen *et al.*, 1999). An immediate hypersensitivity phenomenon is involved in the pathogenesis of oestrosis (Dorchies *et al.,* 1998). *O ovis* larval antigens may possibly be processed by mucosal Langerhans cells, which stimulate T lymphocytes and the

resultant recruitment of mast cells, eosinophils and an increased production of IgE. IgE results in the known allergic reactions via degranulation of mast cells releasing histamine. Histamine increases the permeability of mucosal vessels leading to oedema and copious production of mucus.

The intensity of the cellular response, measured as the number of eosinophils, mast cells and globular leucocytes were found to be correlated to the larval burden albeit with a high variance of cell numbers between sheep (Nguyen *et al.,* 1999). The reactive cells are mainly found in the chorion layer, subjacent to the epithelium and the eosinophils are presumed to be responsible for limiting the parasite populations and the mast cells for sustaining the ongoing hypersensitivity reaction. Of note, degranulation of eosinophils has been shown in vitro to be capable of killing the larvae whereas mast cell degranulation does not (Dorchies *et al.*, 1998).

O ovis infestation produces high levels of specific IgA and IgG in the mucus and high levels of circulating specific IgG (Tabouret *et al.,* 2003b). These antibodies in the serum of infested sheep can be detected using double immunodiffusion, indirect hemagglutinin (Bautista-Garfias *et al.,* 1988) and direct enzyme-linked immuosorbant assay with sensitivity and specificity at 97.4 per cent and 97.6 per cent respectively (Goddard *et al.,* 1999). Specific antibodies to the excretory-secretory product (ESP) of larvae were found to be significantly higher in summer months correlating with the maturation of larvae. However the number of larvae present does not correlate with the antibody titre (Tabouret *et al.,* 2001). Furthermore vaccination of lambs with the L3 ESP did not protect against larval establishment but provided an inhibitory effect on larval development (Frugere *et al.,* 2000).

In addition infected sheep develop thickened, hyperplastic or metaplastic epithelia with eosinophilic exocytosis (Tabouret *et al.,* 2003b). Considering hypersensitivity reaction, eosinophilia, the clinical signs and the chronicity, *O* ovis infested sheep is an ideal model to study human eosinophilic driven chronic sinusitis.

3. Post operative haemorrhage and scarring following surgery

Haemorrhage, synechiae and adhesion formation are common complications of endoscopic sinus surgery. Intra-operative bleeding can lead to an obscured surgical field and may increase the rate of complications. Post operative bleeding can lead to middle meatal clot formation, which requires manual debridement. This in addition to causing the patient discomfort can lead to mucosal trauma which in turn can lead to scarring, adhesion formation and further bleeding and disease recurrence (Gall *et al.,* 2002). Adhesions are formed as a result of an inflammatory response caused by trauma and haemorrhage. If blood clots are not removed by fibrinolysis or absorption, the fibrin deposits can initiate an inflammatory response leading to proliferating fibroblasts, scar tissue formation and adhesions between adjacent surfaces (Holtz 1984). Within the peritoneal cavity instillation of dextra, a polymer known to facilitate fibrinolysis has been shown to prevent post operative adhesions between serosal surfaces (Holtz 1984).

3.1 Wound repair and haemostasis

Haemostasis is the first and primary step in repair. The rupture and damage to vessels result in the exposure of platelets to subendothelial collagen. This causes the platelets to aggregate and activates the intrinsic coagulation cascade. In this milieu of thrombin, fibronectin and their fragments the platelets release cytokines and growth factors such as platelet derived growth factor (PDGF), transforming growth factor β (TGF- β), platelet activating factor (PAF), fibronectin and serotonin from α granules. The locally formed fibrin clot at the end of the coagulation cascade acts as scaffolding for migrating cells such as neutrophils, monocytes, fibroblasts and endothelial cells. These cells are the hallmark of the inflammatory phase, recognised as an essential and early precursor to healing. This phase is characterised by increased vascular permeability, chemotaxis of cells form the circulation into the wound and the local release of cytokines and growth factors. Neutrophils have a role in wounds contaminated with bacteria. Activated monocytes become macrophages that have a fundamental role in several aspects of wound healing such as debridement, matrix synthesis, angiogenesis and fibroplasia (Gibran *et al.*, 1994). Current evidence implicate macrophage activation in scar formation because fetal

wounds that result in scar-less healing release less cytokines and lower numbers of active macrophages ((Otuloye *et al.*,1996). Therefore it can be postulated that the degree of inflammation is correlated with the degree of scarring.

Following the inflammatory phase fibroblasts and endothelial cells continue to be active while the inflammatory neutrophil and monocytes are removed from the site. This leads to the proliferative phase. These cells play a crucial role in repair and are activated by cytokines and growth factors released by platelets and active macrophages. The fibrin clot acts as a source of platelets and macrophages and is responsible for the continuous supply of cytokines (Witte and Barbul 1997).

The third and surgically most important phase in wound healing is the maturation and remodelling phase because this phase determines the strength and character of the scar. During this phase the wound matrix undergoes a change in composition from fibrin and fibronectin to collagen. The amount and type of collagen fibres, the rate and total amount deposited determine the strength and character of the scar. The net collagen synthesis is increased for up to 5 weeks after wounding (Madden and Peacock 1968) and after 3 months the scar will have approximately 80% of the strength of an unwounded site (Levenson *et al.,* 1965). Figure 2 is a schematic diagram of wound healing.

The role of the blood clot in wound healing and scar formation has become clear with in vivo and in vitro studies. Initially the clot provides plasma fibronectin and numerous platelet derived cytokines and growth factors to activate migrating cells initiating inflammation and proliferation. This inturn leads to collagen synthesis. In addition the clot acts as a scaffold for migrating cells and provides an initial framework for the deposition of matrix. Thus, if there is a blood clot within the nasal cavity following endoscopic surgery, it may lead to enhanced inflammatory and proliferative phases resulting in scarring and adhesion formation. Therefore if blood clot formation is prevented, there will be less inflammation and less scarring which prevents adhesion formation. Figure 3 shows wound healing affected by a thrombus.

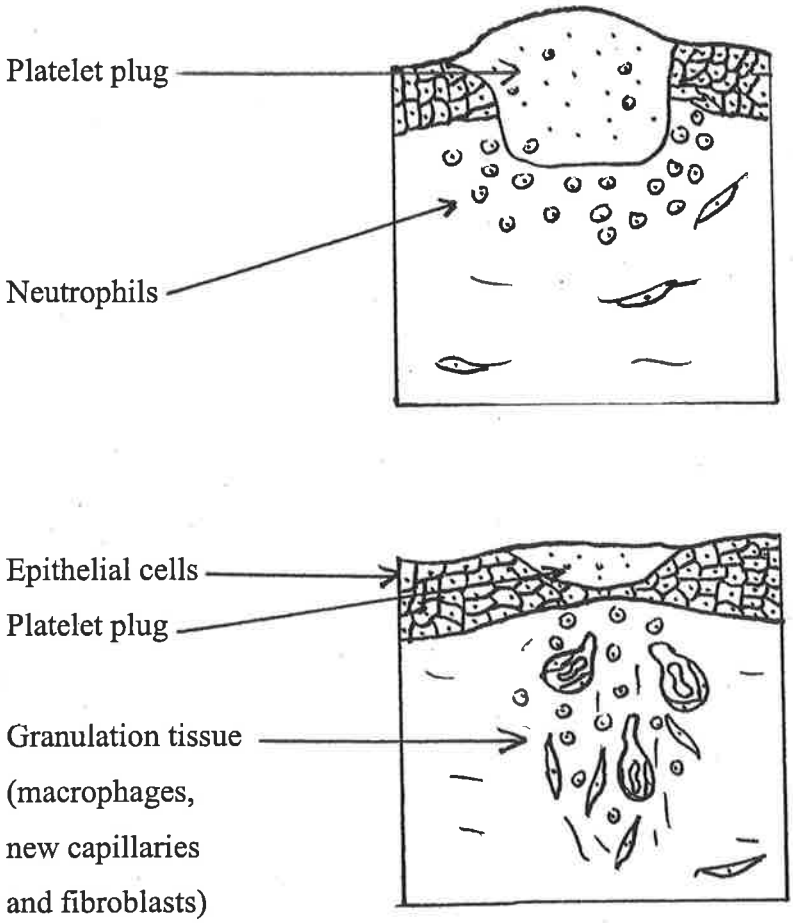


Figure 2: A schematic diagram of wound healing

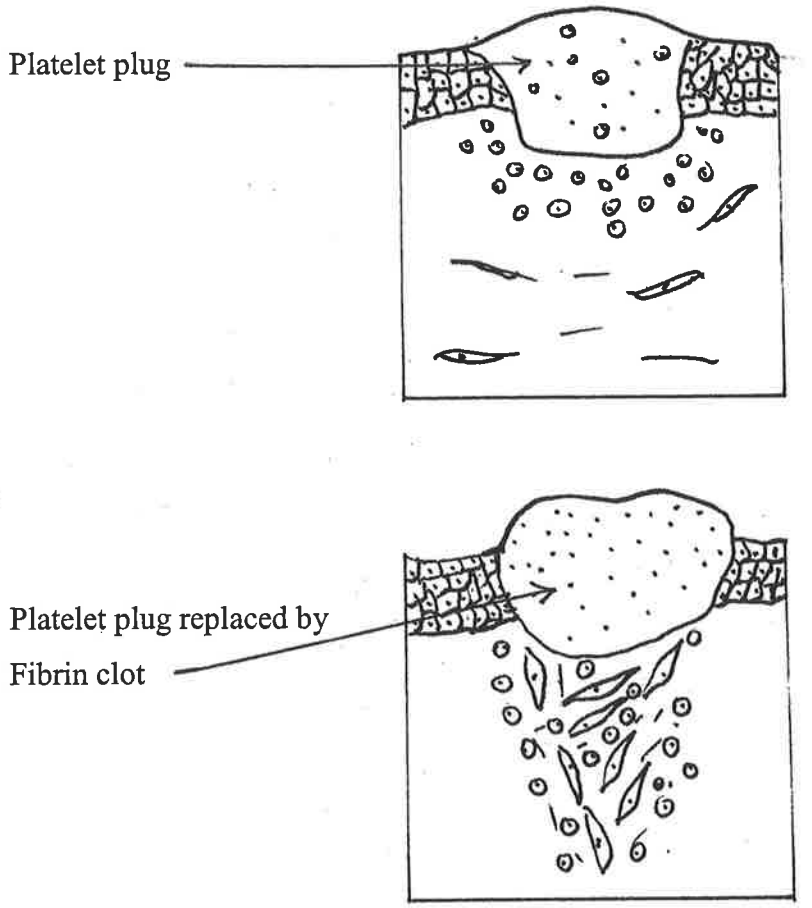


Figure 3: Wound healing affected by a thrombus

3.2 Intra nasal packs

Currently various intranasal packs are used to control post operative bleeding and to prevent blood clot formation within the nasal cavity. Intranasal packing is criticised by many due to the patient discomfort, poor control of bleeding and complications related to the pack (Gall *et al.*, 2002). However packs impregnated with bioactive material may have the potential to significantly improve healing in the nasal mucosa and to prevent adhesion formation (Cowin *et al.*, 2002). Dissolvable packs have been used, eliminating the need for removal and the trauma related to removal. A dissolvable pack impregnated with hyaluronic acid has been shown to improve the rate of re epithelialisation in the nasal mucosa following full thickness mucosal injuries created on the sheep lateral nasal wall (McIntosh *et al.*, 2002). In addition, the height of the epithelial columnar cells was significantly higher in the side treated with hyaluronic acid compared to the control side by day 28 post surgery. The epithelial height returned to that of the control side by day 84 post surgery. This was seen in the absence of inflammatory cells. However at any time point, no significant effect was seen on the rate of re-ciliation. Ciliation was not complete even by day 112 post surgery. Hyaluronic acid may have stimulated cell proliferation and migration of epithelial cells but not differentiation to functional ciliated cells (McIntosh *et al.*, 2002). These findings are consistent of that of Moriyama *et al* (1996) who showed that complete ciliation in the nasal mucosa may take months following full thickness mucosal injuries. Detailed study of healing in the sheep model following full thickness and partial thickness injuries to the mucosa has shown that re-epithelialisation is a slow process but re-ciliation is even slower. This slow process may provide the opportunity to modify the healing process and prevent adhesion formation (Cowin *et al.*, 2002).

Some have questioned the need to place packs within the nose following endoscopic surgery (Orlandi and Lanza 2004). They argue that if suitable measures are taken pre and intra-operatively to prevent bleeding then there is no need for packing following ESS. However this study did not follow up the patients over a period of time sufficient to comment on any effects on packing on adhesion formation.

3.3 Haemostatic agents in endoscopic sinus surgery

Fibrin tissue adhesives are believed to improve mucosal healing and to decrease scarring and synechiae (Gleich *et al.,* 1995). Fibrin tissue adhesive (FTA) consists of concentrated human (autologous) fibrinogen and factor XIII reconstituted with commercially available thrombin. When applied to raw surfaces of sinuses, ostia and middle turbinate post operatively in human subjects, FTA was shown to provide a dry surgical field with no bleeding seen up to 2 days following surgery. On further follow up the FTA treated side appeared to have less crusting compared to the control side which was treated with gelfoam. Furthermore, the patients reported that FTA treated side felt more patent compared to the control side. In addition, a thin clear coating of FTA was seen on the wound surfaces up to 2 weeks after application. The FTA treated side had a tendency toward fewer adhesions and faster healing (Gleich *et al.,* 1995). In addition fibrin sealant (FTA) impregnated with clindamycin and ciprofloxacin has been shown to reduce the rate of goblet cell hyperplasia, mucosal ulceration and inflammation when instilled in rabbit maxillary sinuses chronically infected with *Streptococcus pneumoniae* (Schlosser *et al.,* 2000). Hence fibrin sealants can be used as biodegradable carriers of antibiotics, growth factors or other compounds that aid the healing process.

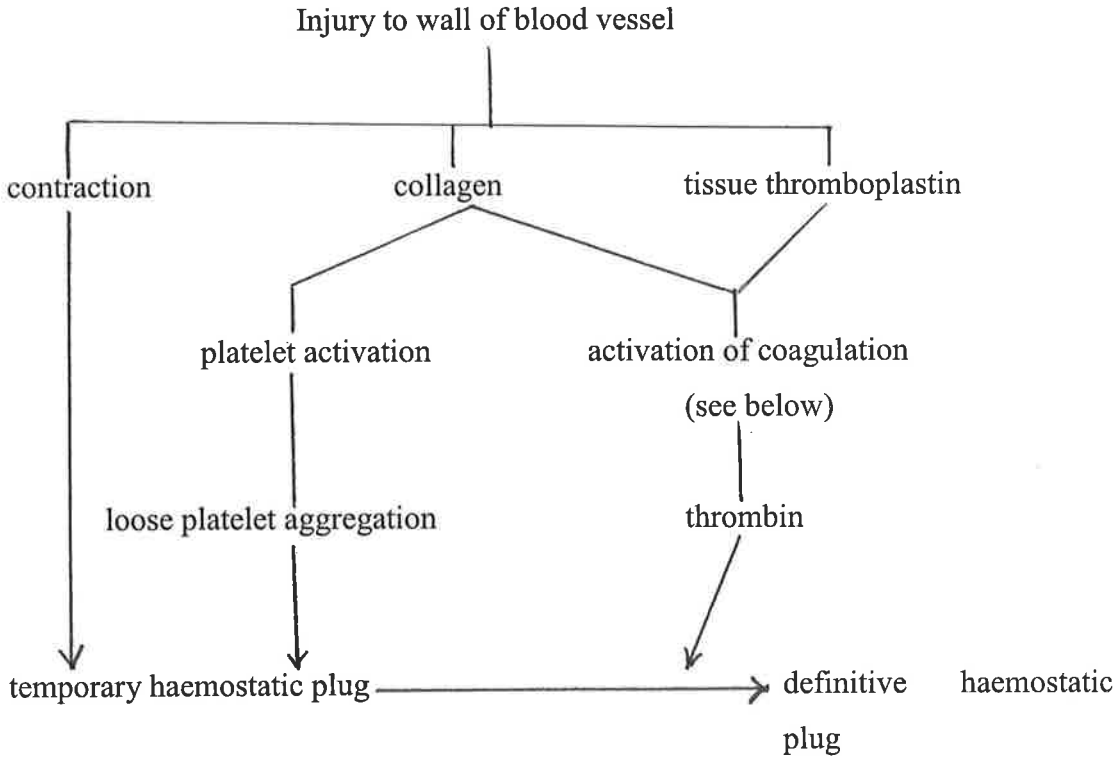
An absorbable granular pack of gelatine matrix and thrombin (FloSeal, Fusion Medical Technologies, Inc. CA) has been shown to effectively reduce bleeding in endoscopic sinus surgery (Gall *et al.,* 2002). FloSeal (bovine collagen) was reconstituted with commercially available thrombin prior to application on to the ethmoid, middle meatus or septum of patients during ESS. They also report that up to 30 days following surgery, 94% of the patients treated with FloSeal appeared to have normal scarring with no adhesions. The degree of bleeding prior to and after the application of FloSeal was evaluated using a 5 point visual analogue scale. In all patients treated with FloSeal, total haemostasis was achieved within 2 minutes in all sites. In this study, all sites in all patients were treated with FloSeal and thus had no control to compare the result with. Therefore no comment can be made regarding the statistical significance of their result. In a randomised controlled clinical study using FloSeal (Baxter Healthcare Corp, IL) using bovine collagen and bovine-derived thrombin was used as a heamostatic agent in children following adenoidectomy

(Mathiasen and Cruz 2004). The time to achieve haemostasis and the degree of bleeding following pack removal (4 point visual analogue scale) were compared with control patients who received cautery. They showed that FloSeal significantly reduced the time for haemostasis and blood loss providing a clear surgical field and easier operation.

In another study FloSeal was found to be comparable to dissolvable hyaluronic acid based packs in terms of stenoses and synecheae (Baumann and Caversaccio 2003). However FloSeal has been shown to significantly increase the rate of adhesions and granulation tissue compared to thrombin-soaked foam (Chandra *et al.*, 2003). Another haemostatic agent CoStasis, which is bovine fibrillar collagen, thrombin and autologous platelet mixture has been successfully applied endoscopically to treat metastatic cancers in the upper gastrointestinal tract (Milkes *et al.,* 2002) but has never been used in the nasal cavity.

3.3.1 Haemostasis

Haemostasis is a result of 2 dynamic cascades, namely the coagulation cascade and the fibrinolytic system. The end product of coagulation is thrombin, and the end product of the fibrinolytic system is plasmin. The major role of thrombin is to initiate formation of fibrin clot by cleaving specific peptide bonds in the plasma protein fibrinogen. Plasminogen (MW 81,000) is converted to plasmin by tissue plasminogen activator (tPA). Plasmin breaks down clot by hydrolysis of peptide bonds in the fibrin molecule. Both plasmin and thrombin are serine proteases and plasma components have been identified that are capable of inactivating them. These suppress the coagulation cascade and the fibrinolytic system within the circulation. Figures 4 and 5 depict the coagulation and fibrinolytic pathways.



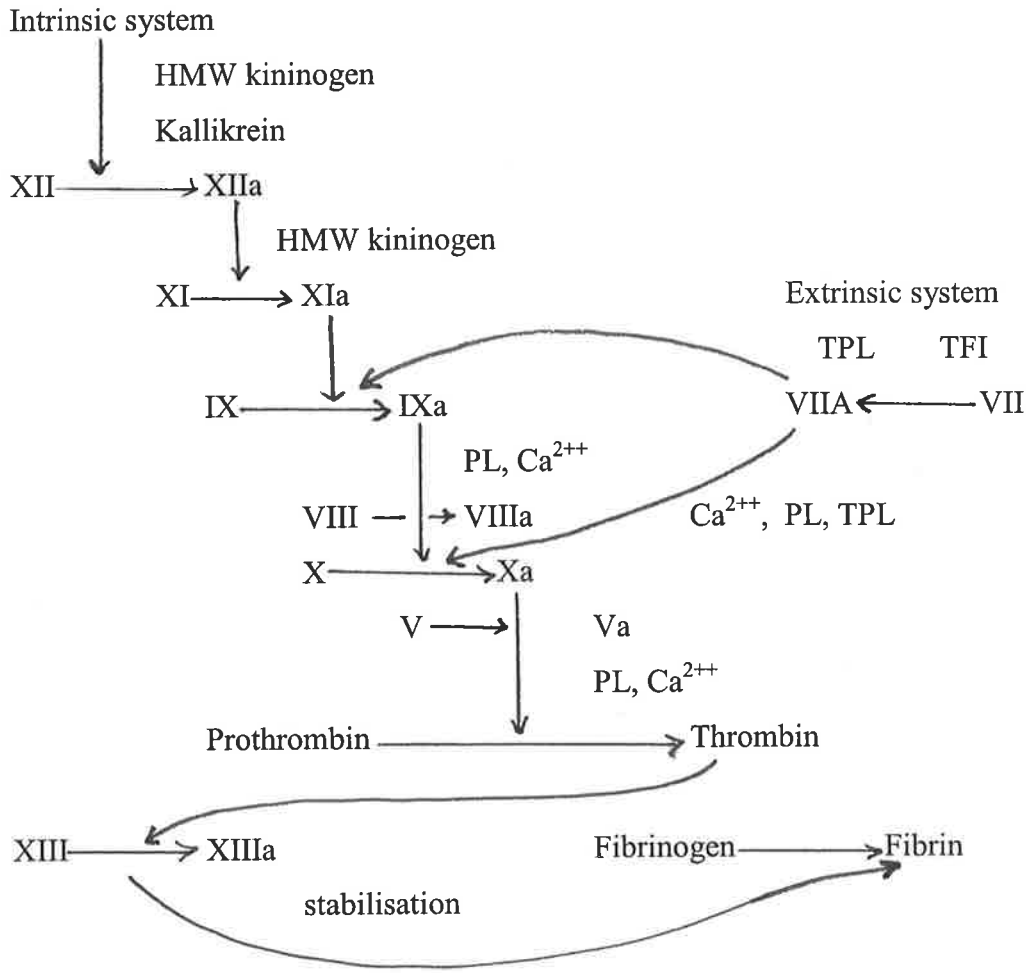


Figure 4: Coagulation cascade (see next page for legend)

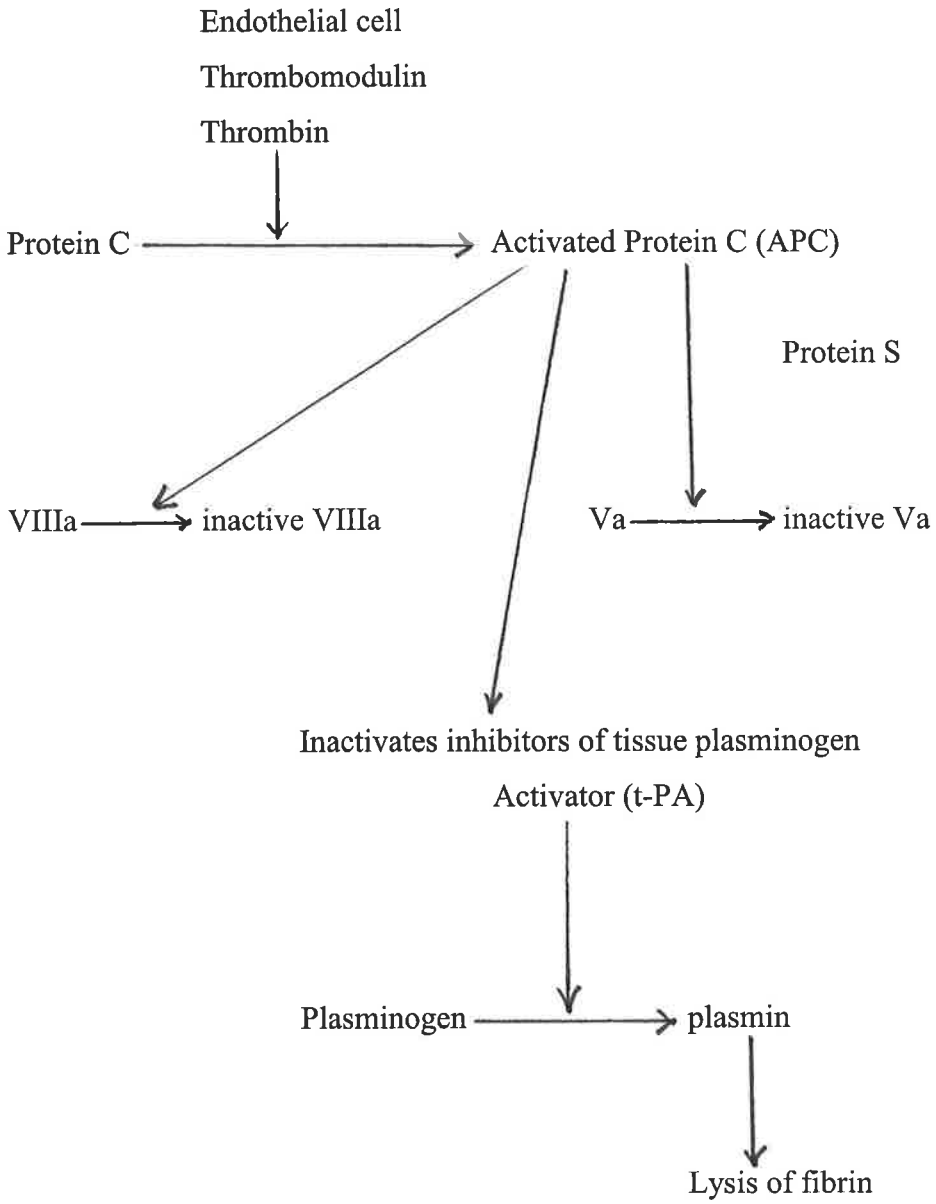


Figure 5: Fibrinolytic pathway

PL = platelet phospholipid

TPL = tissue thromboplastin

TFI = tissue factor pathway inhibitor

HMW = High molecular weight

3.3.2 Epsilon Amino Caproic Acid (AMICAR)

The major inhibitor of the fibrinolytic system within the circulation is antiplasmin (MW 67,000). Plasmin is rapidly neutralised by the formation of 1:1 complexes with antiplasmin via the serine active centre in plasmin. In addition the N terminal 5 triple loop structures or the kringle region is important for binding to fibrin.

Two synthetic derivatives of the amino acid lysine, 6-aminohexanoic acid (aminocaproic acid or AMICAR) and 4-(aminomethyl)cyclohexanecarboxylic acid (tranexamic acid) have antifibrinolytic activity in humans. Both these amino acids reversibly bind to the kringle regions of plasminogen resulting in a marked conformational change in the molecule. This significantly limits the ability of plasminogen to be activated to plasmin. Within the circulation AMICAR can reduce the rate of plasmin-antiplasmin interaction by 10 to 50 fold. This makes more plasmin available in its active form and a rapid breakdown of clot as it is formed within the circulation. This prevents clot formation within the circulation, preventing thrombolic and embolic events. But added to a surface wound, AMICAR inhibits the fibrinolytic system by binding to plasminogen. This prevents the breakdown of minute clots and platelet plugs formed at the cut ends of vessels which leads to a lower rate of bleeding and prevents the accumulation of blood and fibrin degradation products within the wound cavity. This may lead to reduced rates of inflammation and matrix deposition which inturn may prevent scarring and adhesion formation. Figure 6 schematically represents how AMICAR prevents fibrinolysis

Epsilon amino caproic acid (AMICAR) has been extensively used as a hemostatic agent orally in haemophilic patients following dental extractions and oral surgery (Walsh *et al.,* 1975). It is currently available as an intravenous infusion for the use in cardiopulmonary bypass surgery (Greilich *et al.,* 2003), repair of congenital heart defects (Ririe *et al* 2002), orthopaedic surgery (Amar *et al.,* 2003), angioedema (Ritchie 2003), aneurysmal subarachnoid haemorrhage (Roos *et al.,* 2003), cranial arteriovenous fistulae (Kallmes *et al.,* 2000). AMICAR has been used topically combined with 2% carboxypolymethylene gel (Carbopol) in a rabbit model with traumatic hyphema, and was shown to reduce the incidence of secondary haemorrhage (Allingham *et al.,* 1988). Furthermore the concentration of AMICAR

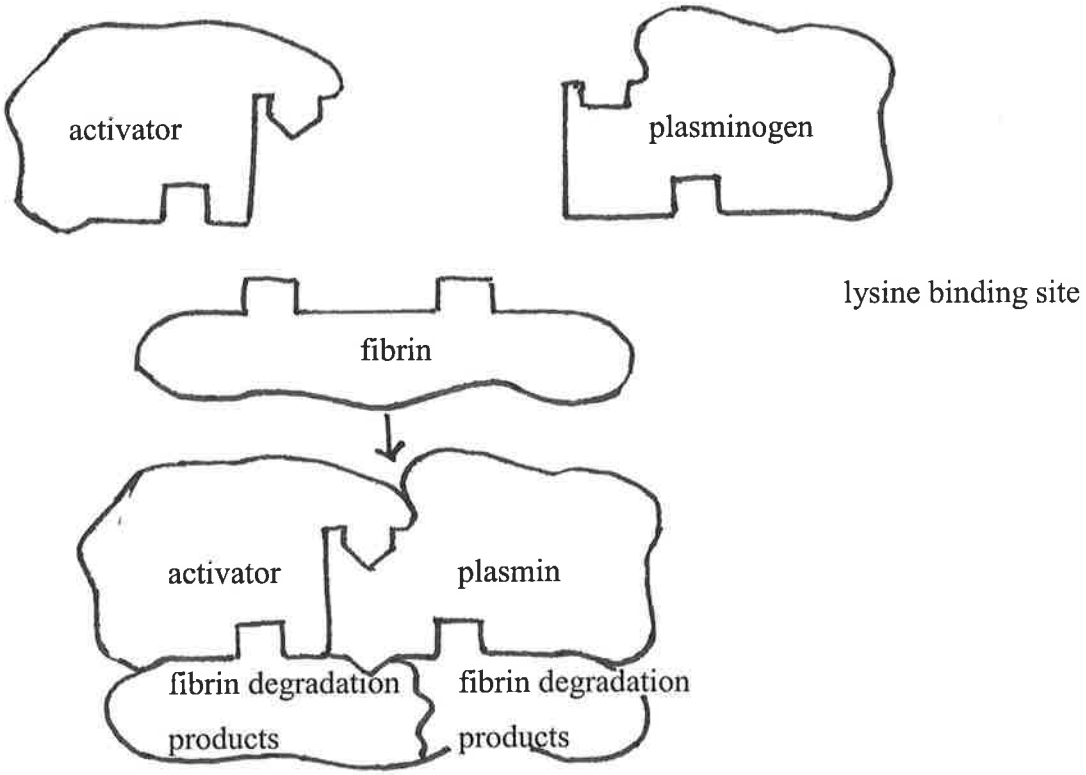
within the aqueous humour after topical administration was shown to be comparable to that achieved by systemic administration (Allingham *et al.,* 1987). In a prospective, randomised double blind multicentre study, AMICAR used topically was shown to be as effective as systemic administration in the prevention of secondary haemorrhage in traumatic hyphema. Furthermore no side effects were observed when AMICAR was used topically (Crouch *et al.,* 1997).

The other synthetic derivative of lysine, tranexamic acid is also an effective topical haemostatic agent. Recently topical intranasal tranexamic acid has been used with considerable success in a hemorrhagic telangiectasia patient controlling bleeding as soon as it started and successfully maintaining the patient's haemoglobin for a number of years (Klepfish *et al.,* 2001). It has also been used successfully in the treatment of epistaxis in haemorrhagic telangiectasia decreasing the incidence of epistaxis by 50% and resulting in the normalization of the patient's haemoglobin (Sabba *et al.,* 2001).

AMICAR is an aqueous solution which can be sprayed onto the surface of a wound and therefore is a good candidate for a topical haemostatic agent. Following endoscopic sinus surgery large mucosal areas may be denuded with the removal of polyps and air cells. Although large blood vessels are not affected this area may ooze over time leading to a significant degree of bleeding and clot formation within the nasal cavity. With the use of topically applied AMICAR small platelet plugs and fibrin clots at these points of ooze may be prevented from being dissolved by the dynamic fibrinolytic activity and potentially preventing further ooze and the accumulation of a large clot within the nasal cavity. This process also has the potential to prevent adhesion formation. In order to prolong the contact of AMICAR with the mucosal surface, a mucoadhesive can be added to the solution.

The following study was conducted to evaluate the effects of topically applied AMICAR on haemostasis over the nasal mucosa and adhesion formation following endoscopic sinus surgery. Reepithelialisation and reciliation of the nasal mucosa was also studied over a period of time to identify any effects of AMICAR on wound healing in the nasal mucosa. The above parameters were also observed when AMICAR was used in combination with mucoadhesive methyl cellulose.

Activation of fibrinolysis



Inhibition of fibrinolysis

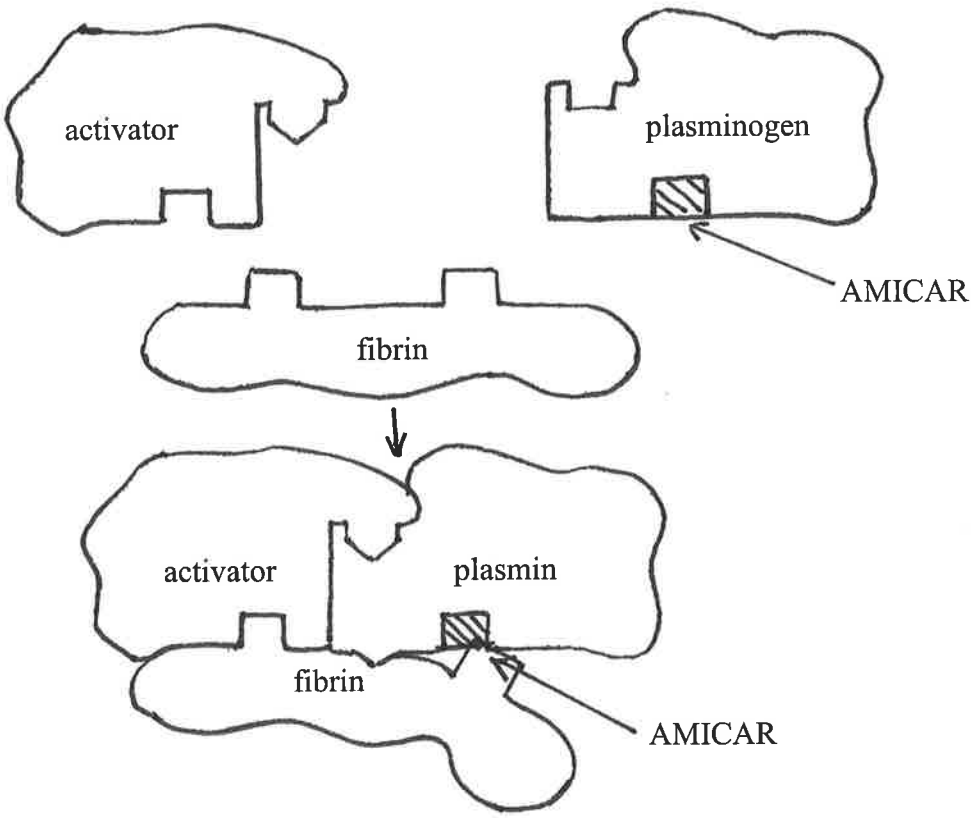


Figure 6: Mechanism of action of AMICAR (amino caproic acid)

METHODOLOGY

The following study was approved by The Queen Elizabeth Hospital Animal Ethics Committee. All animals were managed in accordance with the guidelines set by NH&MRC for the care of experimental animals.

Based on previous findings of a 70%+/-4.4% (SEM) rate of epithelialisation by 56 days following full thickness injuries to the sheep nasal mucosa (Shaw et al 2001b) this study aims to demonstrated a 25% improvement n the rate of reepithelialisation. A power of 80% and a significance level of 0.05 were chosen.
The formula for the power study:

$$n \geq [(u + v)(\sigma_1^2 + \sigma_0^2)] / (\mu_1 - \mu_0)^2$$

where:

$\mu_1 - \mu_0$ = difference between the two sample means
σ_1, σ_0 = standard deviations
u = one sided percentage point of the normal distribution corresponding to 100% - the power
v = percentage point of the normal distribution corresponding to the (two-sided) significance level

Applying this formula with:
Power = 80%
Significance level = 0.05
Aim to demonstrate a 25% improvement of re-epithelialisation
yields:

$$n \geq [(0.84 + 1.96)^2 (2 \times 13.2^2) / (87.5 - 70)^2$$

$$n \geq 8.921$$

Therefore a minimum of 9 sheep were required and 10 sheep were used for the study. Each sheep was its own control using both nasal cavities.

The following study was undertaken as a pilot study to examine trends in the results rather than to seek statistical significance.

1. Inclusion criteria

The subjects for this study were sheep suffering from chronic eosinophilic sinusitis, as a model for endoscopic surgery in the setting of chronic sinusitis. The ectoparasite *Oestrus ovis* causes an eosinophilic sinusitis with local inflammation and symptoms equivalent to human chronic sinusitis.

Sixteen sheep were sedated using 0.5ml xylazine (Ilium xylazil -20; 20mg/ml xylazine as HCl, Troy Laboratories pty Ltd, NSW, Australia) intramuscularly at the IMVS animal holding facility. They underwent a rigid endoscopic examination of the nasal cavities for signs of infestation with *Oestrus ovis*. The signs of live *O ovis* larvae, thick green-yellow mucus, crusting around the nostrils, oedematous mucosa and nasal discharge were sought. Ten sheep with any of the above signs were selected. Five sheep treated with oral ivermectin three months prior were used as controls for *Oestrus ovis* infestation. These sheep were housed at the IMVS animal holding facility and transported to and from the animal laboratory at the Queen Elizabeth hospital as needed.

2. Standardisation Procedure

All sheep in the study underwent the standardisation procedure following the protocol developed by the Department of Otolaryngology and Head and Neck surgery of The Queen Elizabeth Hospital. This standardisation procedure, total middle turbinectomy following decongestion was shown not to significantly affect the mucociliary clearance rate or the histology of the nasal mucosa (Shaw et al 2001). Therefore

following middle turbinectomy the histology and function of the sheep nasal cavity is comparable to the norm.

General anaesthesia was induced using 15ml sodium thiopentone (940mg/g) as 5% solution in normal saline administered via the external jugular vein. The sheep were then intubated in a supine position in a specially designed cradle with the nasal cavity in a vertical orientation and draped. Anaesthesia was maintained with 2% halothane and 4L oxygen per minute. Figure 7 shows the sheep positioned in the cradle, in a supine position prior to the operation.

Local anaesthesia of the nasal cavities were achieved using 4 puffs of Cophenylcaine spray (ENT Technologies, WA, Australia) (5% lignocaine HCl, 0.5% phenylephrine HCl, 0.1mg/ml benzylkonium Cl) in each nostril followed by an infiltration of mucosa with 3ml of 2% Xylocaine with 1;80,000 adrenaline over the attachment of the middle turbinate. Subsequently neuropatties soaked in 10% cocaine solution were applied superior, inferior and medial to the middle turbinate on each side and left for 10 minutes. The above procedure ensures haemostasis as well as local anaesthesia and is similar to that performed in human sinus surgery.

Following the removal of neuropatties the nasal cavity was examined using a 0 degree 4mm rigid Hopkins rod and a 30 degree 4mm Richards Sinuscope, attached to a Storz Endovision light source and camera system and Sony PVM Trinitron monitor. Figure 8 is shows these instruments. The middle turbinate was dissected from the lateral nasal wall to its posterior extent along its attachment to the lateral nasal wall using endoscopic turbinate scissors. The lateral nasal wall and the septum were carefully preserved. Upon the removal of the turbinate, neuropatties were replaced in the nasal cavity to achieve haemostasis. Further haemostasis of the posterior aspect was achieved using unipolar suction diathermy. Following satisfactory haemostasis the anaesthesia was ceased and the sheep extubated. The animals were observed for 4 to 5 days at the TQEH animal facility for pain, bleeding or any other signs of distress, and then sent to IMVS animal holding facility.

Figure 7: sheep positioned supine in the cradle prior to operation

Figure 8: Instruments and equipment set up

Figure 9 shows a coronal view of the sheep skull following middle turbinectomy indicating the locations of the mucosal injuries. Figure 10 shows the endoscopic appearance following middle turbinectomy. Note the numerous *Oestrus ovis* larvae.

Figure 9 : Parasagittal CT of sheep head

Figure 10 : Axial CT of sheep with the middle turbinates intact

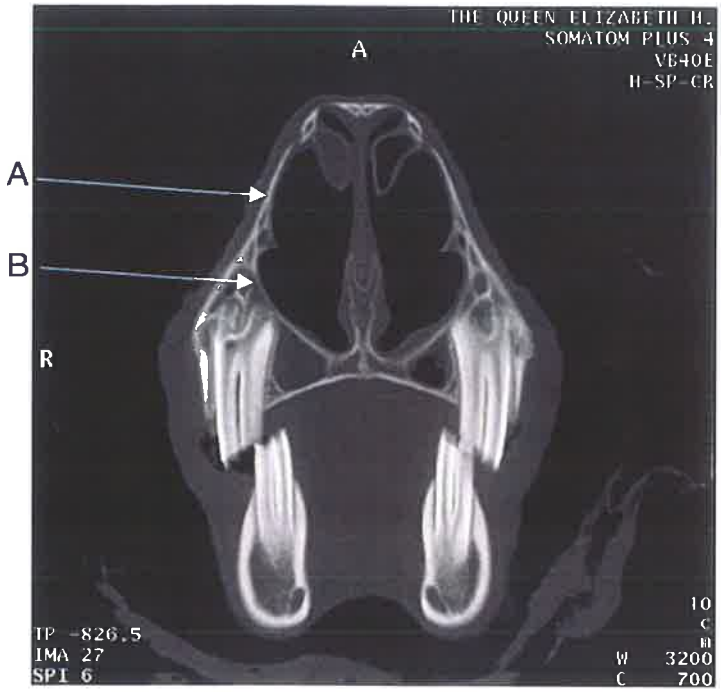


Figure 9: Coronal CT view of the sheep skull. The middle turbinates have been removed. Full thickness mucosal injuries were created on the areas labelled A and B. A = superior to the attachment of middle turbinate. B = inferior to the attachment of middle turbinate.

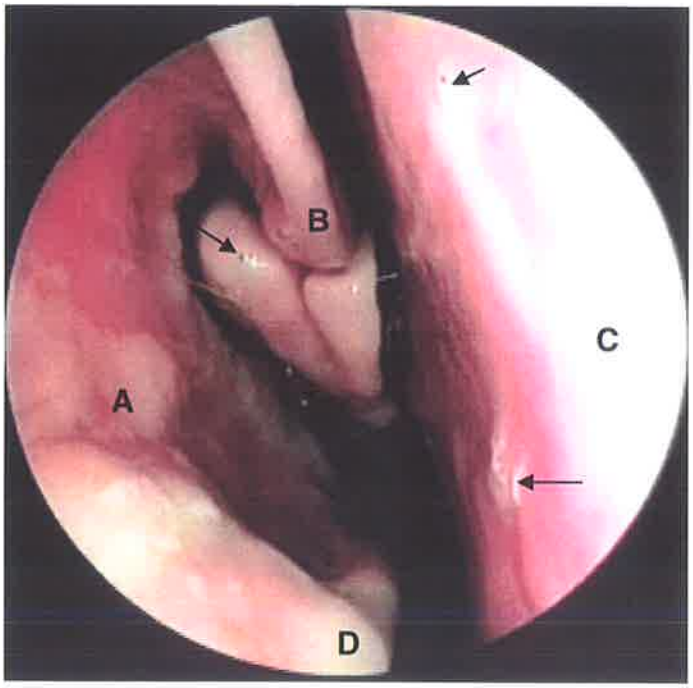


Figure 10: Endoscopic view post middle turbinectomy. A = lateral nasal wall superior to the attachment of the middle turbinate (D);, B = ethmoturbinals;, C = nasal septum. Arrows indicate *Oestrus ovis* larvae.

3. Initial mucosal injuries and the use of AMICAR®

The sheep were brought back to TQEH animal facility 4 to 6 weeks following the turbinectomies. They were anaesthetised as above. The nasal cavity was examine endoscopically to ensure healing of the middle turbinate attachment and any excess mucus or mucopus removed by suction.

For day 0 biopsies, mucosal incision of about 0.5 mm was made using a Freer's elevator and elevated. Two biopsies were obtained from the elevated mucosa using an endoscopic biopsy forceps. This procedure was performed bilaterally.

Initial full thickness mucosal injuries were performed over the lateral nasal wall both superior and inferior to the middle turbinate attachment, circumferentially over the maxillary ostium and the lateral surface of the ethmoturbinals and adjacent lateral nasal wall using a microdebrider fitted with a 4mm cutting blade (Xomed, Jacksonville, Fl). The lateral nasal wall injuries were commenced at 15mm posterior to the aperture of nostrils and extended posteriorly to about 10mm form the anterior aspect of the ethmoturbinals. These measurements were made using an intranasal measuring device. Figure 11 is a photograph of the instruments used for the biopsy procedure.

The side to receive the haemostatic agent was randomly selected using a random number table, with odd numbers being the left nasal cavity and even numbers being the right nasal cavity. In sheep numbers 1 to 5, a total of 6ml of AMICAR® (epsilon amino caproic acid, 250mg/ml iv preparation, Xanodyne Pharmacal Inc, Florence, KY) was sprayed as a mist immediately after injury. Approximately 3ml were sprayed over the lateral nasal wall and 3ml over the maxillary ostium and the ethmoturbinal. In sheep numbers 6 to 10, a total of 5ml of AMICAR mixed with 5 ml of mucoadhesive methyl cellulose (0.6% in 6% phosphate buffered saline pH 6.8) was sprayed on the test side. Methyl cellulose is viscous and lipophilic, and has the ability to adhere to mucosal surfaces. This was used to ensure enhanced contact between AMICAR and the mucosal surface and to prolong the time of contact. On the contralateral side the initial injuries were performed as described, 6ml of normal

saline was sprayed as a mist and the surgical field bleeding was graded at 2 minute intervals.

The degree of bleeding in the 4 sites was graded immediately after the injury and at 2 minute intervals until haemostasis was achieved according to the scale shown in figure 12. The total time to achieve complete haemostasis was recorded for each wound on each side. The animals were observed in the laboratory for the following 3 days.

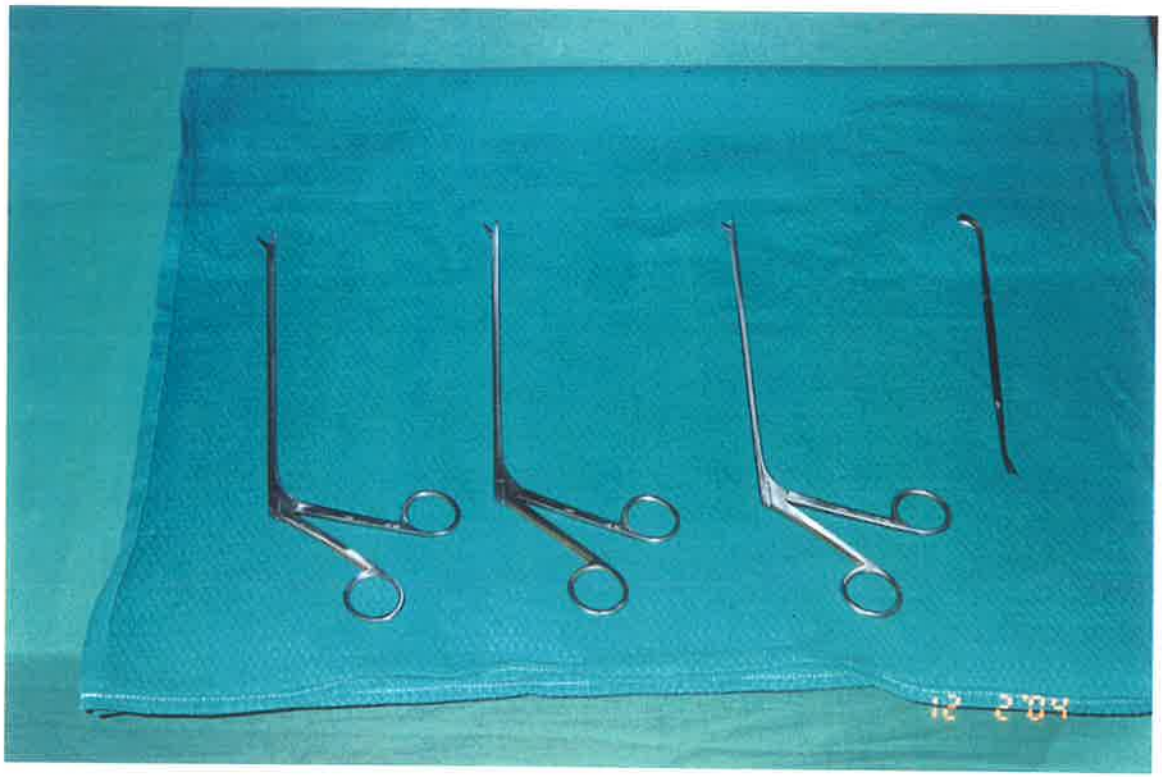


Figure 11. Instruments used for the biopsy procedure

SCALE FOR GRADING SURGICAL FIELD BLEEDING
Sheep no _____
Date _____

0 mins
0 1 2 3 4 5 6 7 8 9 10

2 mins
0 1 2 3 4 5 6 7 8 9 10

4 mins
0 1 2 3 4 5 6 7 8 9 10

6 mins
0 1 2 3 4 5 6 7 8 9 10

8 mins
0 1 2 3 4 5 6 7 8 9 10

10 mins
0 1 2 3 4 5 6 7 8 9 10

Total time to complete haemostasis-

0 –no bleeding
1- 1-2 points of ooze
2- 2-4 points of ooze
3 – 4-6 points of ooze
4- 6-8 points of ooze
5 – 8-10 points of ooze
6 - >10 points of ooze, obscuring surface
7 - > 10 points of ooze, blood trickling towards posterior choana
8 - > 10 points of ooze, total surface obscured, moderate amounts of blood tricking towards posterior choana, intermittent suctioning or dabbing required
9 – >10 points of ooze, obscured surface, bleeding down to choana requiring suction
10 – constant suctioning required

Figure 12 : Visual analog scale for grading the surgical field bleeding

The nasal cavity of the sheep were endoscopically examined on the day following this procedure, under sedation using 0.1 – 0.2ml xylazine (20mg/ml) and the appearance of the injured surface, any bleeding or crusting were recorded. This was mainly to ensure that haemostasis was achieved and no bleeding continued from the denuded surfaces of the nasal cavities.

4. Demonstration of eosinophilia in the epithelium and mucin

The following study was performed to confirm the diagnosis of eosinophilic sinusitis. Prior to initial injuries in the test sheep and in the control sheep, nasal mucosal brushings were obtained using a cytobrush. The brushings were collected into 1ml phosphate buffered saline and centrifuged at 1g for 10mins. The supernatant was discarded and the cell pellet was smeared onto 2 microscopy slides. These were air dried for 24 hours and then stained with haematoxylin and eosin. They were then coverslipped with a mounting medium. Cell smears were examined under high power (magnified 1000 times) with a light microscope. Three fields with a single cell thickness were selected and the eosinophils were counted in them. These were averaged for each side in each sheep.

Using the day 0 biopsies, the epithelium was examined under high power (x1000) with a light microscope. Three fields per section were selected on 2 sections per left or right side of the sheep. The number of eosinophils seen within the epithelium per field was counted and the average number of eosinophils per field was calculated for left and right side of each sheep.

5. Biopsies for evaluation of reepithelialisation and reciliation

Serial biopsies were planned to evaluate the healing process of the nasal mucosa over the injured lateral nasal wall, and to examine any effects of AMICAR on the healing

process. The rate of healing at 28 day intervals was evaluated by measuring the degree of epithelialisation and re-ciliation.

Biopsies of the lateral nasal wall mucosa (over the initially injured area) were performed under sedation using 0.1-0.2ml xylazine (20mg/ml) intramuscular administration and local anaesthesia with cophenylcaine spray. Biopsies were performed on day 0 (immediately prior to injury as described), day 28 (anterior inferior lateral nasal wall), day 56 (posterior inferior lateral nasal wall), day 84 (posterior superior lateral nasal wall) and on day 112 (anterior superior lateral nasal wall). In the control sheep only initial biopsies were taken from the lateral nasal wall for the evaluation of eosinophils. They were returned to the IMVS animal facility following this. No injuries were performed on these 2 sheep. In all the experimental sheep 2 specimens were obtained from each site at each visit. One of the biopsies was placed in phosphate buffered saline and the other placed in 10% formaldehyde.

6. Preparation and Analysis of Light Microscopy Specimens

The following protocol described by Shaw et al (2001) and McIntosh et al (2002) has been shown to produce reproducible and reliable results for the evaluation of re-epithelialisation

. The biopsies fixed in formaldehyde were placed with the mucosal surface up between 2 layers of foam within a plastic cassette and labelled. These were placed in a Tissue-Tek VIP processor for overnight fixation. Subsequently they were mounted in paraffin wax and placed at -4°C for 1 hour. These blocks were then sectioned using a Leica microtome at 0.4 mm and mounted on glass microscope slides. These were air dried overnight and stained with haematoxylin and eosin. They were coverslipped with an adhesive.

Reepithelialisation

The percentage surface covered with epithelium was calculated by measuring the length of basement membrane on the section and the length of epithelium seen on the basement membrane at low power (magnified 20 times), using VideoPro, a computerised image processor. This method was validated by Shaw et al (2001). The vertical distance between the basal membrane and the highest point in the epithelium was considered as the maximum height of the epithelium. In addition 2 other measures were obtained by measuring the height of the epithelium within the same field. The mean of these 3 measures was considered as the average epithelial height.

The percentage epithelial coverage was transformed using an arcsine transformation in order to perform parametric statistics. A Student's T test was performed to analyse the difference between the rates of epithelialisation at day 28, 56, 84 and 112 in each treatment side, and between the 2 treatment sides at each time interval.

A Student's T test was used to analyse the epithelial height and compare the 2 treatment sides at each time interval and between the time intervals.

The rate of reepithelialisation was then correlated with the average number of eosinophils using regression analysis.

7. Preparation and analysis of Electron microscopy specimens

Specimens placed in phosphate buffered saline were sonicated for 20 minutes to remove blood from the surface. They were then transferred to phosphate buffered saline (pH 7.2) with 4% paraformaldehyde and 1.25% glutaraldehyde. These were stored at $4^{\circ}C$, processed using an automated processor and dehydrated sequentially using osmium tetroxide, 70% ethanol, 90% ethanol, 95% ethanol, 100% ethanol, 100% acetone and 100% ethanol (1:1) and finally in 100% ethanol. They were then dried in a CO_2 critical point dryer and then mounted on electron microscopy stubs. A final coating of gold and carbon was then applied.

Digital images of 3 fields selected as representative fields and devoid of blood and mucus from the epithelial surface were then obtained using a Phillips Field Emission Scanning Electron Microscope Excel 30 and stored in a high resolution TIFF format. The images were obtained at 600x and 3000x. AnalySIS Pro® (Soft Imaging Systems GmbH) (Version 3.00) image analysis software was used to measure the area covered with mature cilia relative to the total area of the field observed. The percentage area covered by mature cilia was recorded for each specimen using images obtained at 600x. The maturity of cilia was evaluated using 3000x images. Immature cilia were identified by their short stubby appearance and the percentage of area covered by immature cilia was recorded. Total area of ciliation was obtained by adding the above numbers. The rate of total ciliation was correlated with the level of epithelial eosinophilia using regression analysis.

8. Assessment of Intranasal Adhesions and Synechiae

At the time of each biopsy the ethmoturbinal, lateral nasal wall and the maxillary ostium were examined endoscopically. This was performed to examine the healing process around the maxillary ostium and the injured mucosa over the ethmoturbinal and adjacent lateral nasal wall. The aim was to establish a relationship, if any, between the use of AMICAR and scarring. The presence of adhesions between the lateral nasal wall and the ethmoturbinal and synechiae around the maxillary ostium were recorded as a positive and the absence of adhesions or synechiae were recorded as a negative. These images were captured in a video cassette attached to the Sony Camera system.

A Fischer's exact test was used to determine correlation between the formation of adhesions, synechiae and the use of the haemostatic agent.

RESULTS

1. Eosinophilia

1.1. Secreted eosinophils in mucus

The average numbers of eosinophils seen per high power field (x1000) in the mucus smears obtained from each side for each sheep are given in table 1.

Table 1. Average numbers of eosinophils in mucus smears per high power field

Sheep	Side	Average number of eosinophils
Control 1	Left	0
	Right	2.166
Control 2	Left	0.33
	Right	0
Control 3	Left	0
	Right	0
Control 4	Left	0
	Right	0
Control 5	Left	0
	Right	0
1	Left	1.16
	Right	4.5
2	Left	1
	Right	60.16
3	Left	2.33
	Right	6
4	Left	8.6
	Right	23
5	Left	27.5
	Right	29.33
6	Left	2.33

Sheep	Side	Average number of eosinophils
	Right	1.83
7	Left	1.67
	Right	1.33
8	Left	41.5
	Right	32.83
9	Left	39.67
	Right	21.83
10	Left	2.67
	Right	6.83

The mean number of eosinophils in the mucus smears in the control animals was 0.5 (SE = 3.8536; SD = 17.2340; 95% CI = 8.0667) while that in the *Oestrus ovis* infested sheep was 16.23 per high power field (SE = 0.2155; SD = 0.6816; 95% CI = 0.4875). This difference was statistically significant at p=0.003 when analysed using a 2 sample t test assuming equal variance. Therefore for the diagnosis of *Oestrus ovis* infestation induced chronic sinusitis in sheep, at least 1.6 eosinophil per high power field, averaged over 3 fields, should be detected in the mucous smear (calculated using the mean eosinophil count for controls + 2 standard deviations). Figure 13 is a light micrograph showing a mucus smear with eosinophils.

1.2. Epithelial Eosinophilia

The average numbers of eosinophils seen per high power field in the initial mucosal biopsies are given table 2.

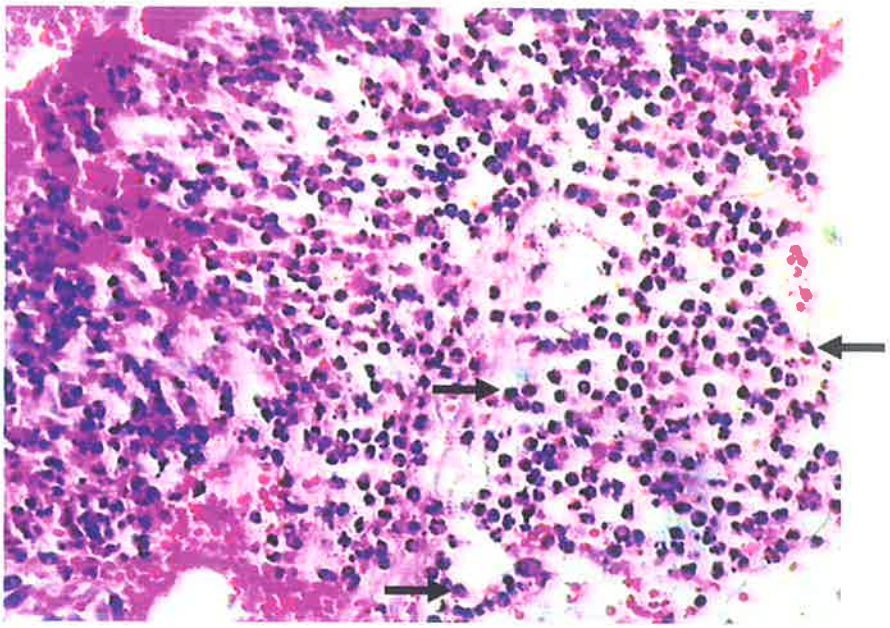


Figure 13: Eosinophils in the mucous smear obtained from the nasal cavity of a sheep infested with *Oestrus ovis*. Several of the eosinophils are indicated by arrows. Haematoxylin and eosin, x20.

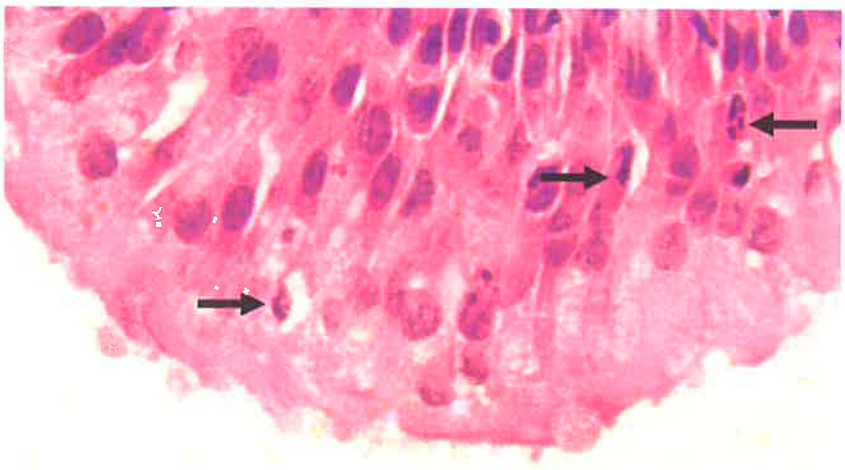


Figure 14: Cross section of the nasal epithelium of a sheep infested with *Oestrus ovis*. Eosinophils among the epithelial cells are indicated by arrows. Haematoxylin and eosin, x1000.

Table 2: The average numbers of epithelial eosinophils per high power field in the initial mucosal biopsies

Sheep	Side	Average number of eosinophils
Control 1	Left	0.21
	Right	N/A
Control 2	Left	0.14
	Right	N/A
1	Left	7.5
	Right	12.5
2	Left	11.4
	Right	15.22
3	Left	23
	Right	3.5
4	Left	0.94
	Right	0.11
5	Left	0.61
	Right	5.6
6	Left	2
	Right	0.88
7	Left	0.88
	Right	0.27
8	Left	2.22
	Right	3.33
9	Left	20.22
	Right	3.11
10	Left	4
	Right	1.39

The mean number of epithelial eosinophils was 0.18 (SE = 0.035; SD = 0.0494; 95% CI = 0.4447) in the control group and 5.84 (SE = 1.556; SD = 6.961; 95%CI = 3.257) in the *O ovis* infested group. A p of 0.05 shows a statistically significant

difference (two sample t test assuming equal variance). Figure 14 is a light micrograph showing epithelial eosinophils.

Therefore for the diagnosis of *Oestrus ovis* infestation induced chronic sinusitis at least 0.3 eosinophils per high power field, averaged over 3 fields should be present in a mucosal biopsy section (calculated using control mean + 2 standard deviations). Figure 15 illustrates the epithelial eosinophilia versus secreted eosinophils in the mucus.

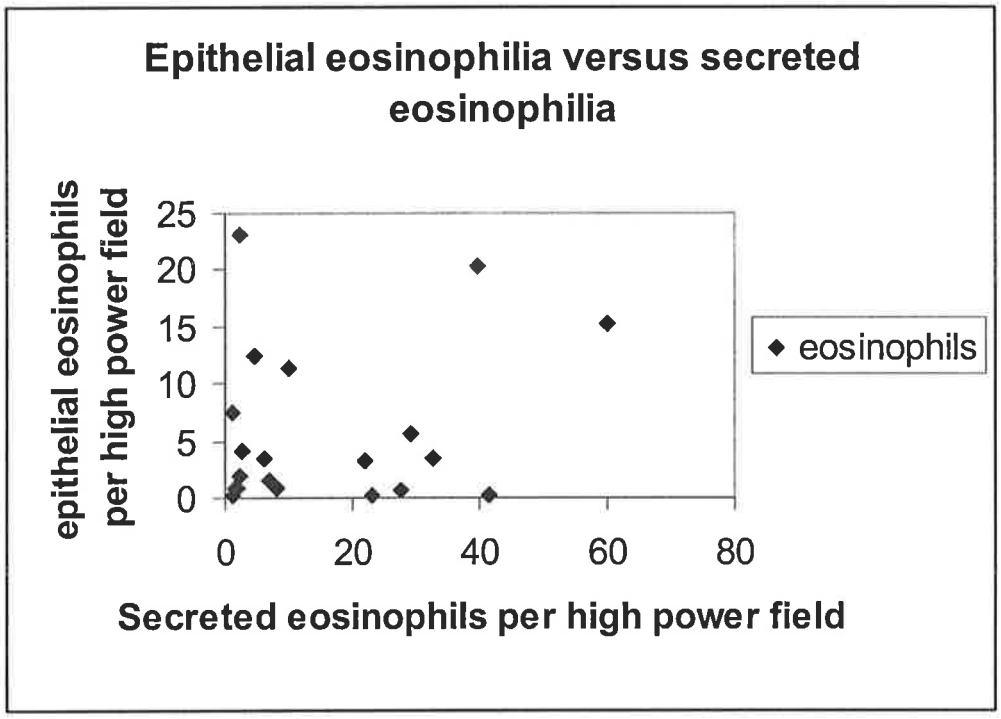


Figure 15: Epithelial eosinophilia versus secreted eosinophils in mucus for each side in each sheep.

Figure 16 shows the relationship between eosinophils numbers in the epithelium and the secreted eosinophil number in each side in each sheep.

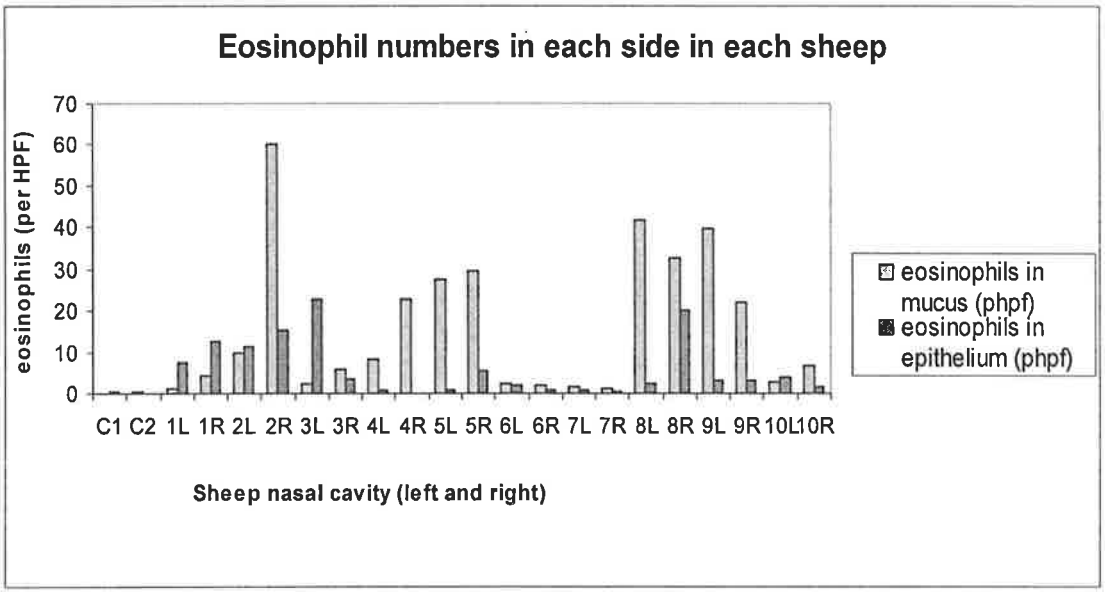


Figure 16: Epithelial and secreted eosinophil number in each side in each sheep

The secreted eosinophils in each side of each sheep were correlated with the corresponding epithelial eosinophils using single factor ANOVA and regression analysis. There was no correlation between these 2 parameters. ($F=1.207$, significance $F= 0.284$; $p=0.018$; $R=0.009$).

2. Degree of Surgical Field Bleeding and Haemostasis

2.1 Grading of surgical field bleeding

The grades of surgical field bleeding for each site at each point of observation are given in tables 3 to 7.

Table 3: Grade of surgical field bleeding in the ethmoturbinals and adjacent lateral nasal wall. AMICAR = amino caproic acid; MC = methyl cellulose

Treatment	Normal saline						AMICAR +/- MC			
Time (mins)/ Sheep number	0	2	4	6	8	10	0	2	4	6
1	10	9	3	4	3	0	10	1	0	
2	5	2	0				10	2.5	1	0
3	4	0					7	4	2	0
4	5	3	2.5	1.5	0		4	0.5	0	
5	9	3	1				5	3	0.5	0
6	4	0					10	5	0	
7	5	2	0				8	2	0	
8	3	0					7	1	0	
9	5	3	2	0			5	2	0	
10	5	2	0				4	2	0	

Table 4: Grade of surgical field bleeding in the maxillary ostium
AMICAR = amino caproic acid; MC = methyl cellulose

Treatment	Normal saline					AMICAR +/- MC			
Time (mins)/ Sheep number	0	2	4	6	8	0	2	4	6
1	4	3	0			2	1	1	0
2	2	0				3	2	1	0
3	1.5	1	1	0		2	0		
4	2	1.5	1	1	0	1.5	1	0	
5	3	1	1	0		2	1	0	
6	5	0				8	4	2	0
7	5	2	0			8	2	0	
8	3	0				7	4	3	0
9	6	3	3	3	0	4	1	0	
10	4	3	0			6	3	0	

Table 5: Grade of surgical field bleeding in the lateral nasal wall superior to the attachment of middle turbinate. AMICAR = amino caproic acid; MC = methyl cellulose

Treatment	Normal saline					AMICAR +/- MC			
Time (mins)/ Sheep number	0	2	4	6	8	0	2	4	6
1	9	6	3.5	0		8	1	0	
2	6	5	2	0		7	4.5	3	0
3	5.5	0.5	0			4	0		
4	7	3.5	2.5	2	0	4.5	2	0	
5	7	5	2	0		5	2	1	0
6	6	1	0			6	3	0	
7	5	2	0			7	0		
8	2	1	0			3	2	0	
9	3	1	0			4	2	1	0
10	3.5	2	1	0		6	2	1	0

Table 6: Grade of surgical field bleeding in the lateral nasal wall inferior to the attachment of the middle turbinate. AMICAR = amino caproic acid; MC = methyl cellulose

Treatment	Normal saline								AMICAR +/- MC						
Time(mins) Sheep #	0	2	4	6	8	10	12	14	0	2	4	6	8	10	12
1	9	6	3.5	4	3	0			8	3	2	2	1	1	0
2	7	5	2.5	2	0				7	4	3	3	2	1	0
3	6.5	3.5	2.5	1.5	1	1	0		4	2	2	2	0		
4	7	5	3	2	1.5	1	1	0	4.5	4	2	1.5	1	0	
5	5	7	5	3	1	1	0		5	4	2	2	1	0	
6	6	3	2	1	0				7	5	2	1	1	0	
7	5	3	1	0					7	2	2	1	1	0	
8	5	4	1	1	0				4	3	0				
9	4	3	3	1	0				5	4	0				
10	4	3	1	1	0				5	2	1	0			

The results of a Wilcoxon sign rank test are given in table 3 below for AMICAR vs normal saline, AMICAR + methyl cellulose vs normal saline and for all sheep (AMICAR with or without methyl cellulose vs normal saline).

Table 7: Grade of surgical field bleeding – Wilcoxon sign rank test
AMICAR = epsilon amino caproic acid, NS = normal saline, MC = methyl cellulose
W= Wilcoxo
n number

Surgical site	AMICAR vs NS		AMICAR + MC vs NS		AMICAR=/- MC vs NS	
	W	p	W	p	W	p
Ethmoturbinals	89.5	0.10	8.0	0.183	151	0.704
Maxillary ostium	80	0.268	11.5	0.062	137.5	0.341
Superior lateral nasal wall	111	0.004	7	0.076	199	0.166
Inferior lateral nasal wall	342	0.002	86.5	0.502	780	0.050

There was a statistically significant reduction in the grade of bleeding in the lateral nasal wall both superior and inferior to the middle turbinate attachment when AMICAR alone was used, compared to normal saline. But when AMICAR was used in combination with methyl cellulose this significance was lost. Furthermore there was a trend towards a reduction in the grade of the surgical field bleeding with the use of normal saline in comparison to AMICAR with methyl cellulose. When data for all sheep were pooled for each site (n=10) using AMICAR with or without methyl cellulose only the lateral nasal wall inferior to the attachment of the middle turbinate showed any significant reduction in the grade of bleeding compared to normal saline.

3. Correlation between secreted eosinophilia and the grade of surgical field bleeding

The correlation between secreted eosinophils and the initial grade of bleeding at the time of the full thickness mucosal injury was calculated for each side in the lateral nasal wall superior and inferior to the middle turbinate attachment. The results are as follows.

Table 8. Correlation coefficients of secreted eosinophils (PHPF) in the lateral nasal wall and the initial grade of surgical field bleeding

LNWS = lateral nasal wall superior to the middle turbinate
LNWI = lateral nasal wall inferior to the middle turbinate

Treatment	Eosinophils and site	Correlation coefficient
Normal saline	Eosinophils/LNWS	-0.41
	Eosinophils/LNWI	-0.34
	Grade for LNW S/I	0.80
AMICAR +/- MC	Eosinophils/LNWS	-0.71
	Eosinophils/LNWI	-0.08
	Grade for LNW S/I	0.9

While there was a high correlation between the grade of surgical bleeding between the superior and inferior regions of the lateral nasal wall, there was no correlation between the number of secreted eosinophils and the severity of bleeding.

4. Correlation between epithelial eosinophilia and the grade of surgical field bleeding

Table 9 shows the correlation coefficients for the grade of surgical field bleeding immediately after the treatment of normal saline or AMICAR with or without methyl cellulose and the average number of eosinophils seen per high power field in the lateral nasal wall. The correlation of surgical field bleeding between the superior and inferior lateral nasal wall is also given.

Table 9: Correlation coefficients of epithelial eosinophils (PHPF) in the lateral nasal wall and the initial grade of surgical field bleeding

LNWS = lateral nasal wall superior to the middle turbinate

LNWI = lateral nasal wall inferior to the middle turbinate

Treatment	Eosinophils and site	Correlation coefficient
Normal saline	Eosinophils/LNWS	0.23
	Eosinophils/LNWI	0.5
	Grade for LNW S/I	0.8
AMICAR +/- MC	Eosinophils/LNWS	-0.1
	Eosinophils/LNWI	-0.04
	Grade for LNW S/I	0.9

While there was a high correlation between the grade of surgical field bleeding between the superior and inferior parts of the lateral nasal wall on both normal saline treated side and the AMICAR +/- MC treated side and the eosinophilia in the epithelium (correlation coefficient 0.8 and 0.9 respectively) there was no correlation between the grade of bleeding and the average number of eosinophils seen in the epithelium.

5. Total time to achieve complete haemostasis

The total time to achieve complete haemostasis for each injured site treated with normal saline or AMICAR with or without methyl cellulose is given in tables 10 to 13.

Table 10: Total time (minutes) to achieve complete haemostasis in the ethmoturbinal and adjacent lateral nasal wall

AMICAR = amino caproic acid; MC = methyl cellulose

Treatment Sheep number	Normal saline	AMICAR +/- MC
1	10	4
2	4	6
3	2	6
4	8	3.5
5	6	5
6	2	4
7	4	4
8	2	4
9	6	4
10	4	3.5

Table 11: Total time (minutes) to achieve complete haemostasis in the maxillary ostium

AMICAR = amino caproic acid; MC = methyl cellulose

Treatment Sheep number	Normal saline	AMICAR +/- MC
1	6	6
2	8	6
3	6	4
4	8	4
5	8	6
6	2	6
7	4	4
8	2	6
9	6	4
10	4	4

Table 12: Total time (minutes) to achieve complete haemostasis in the lateral nasal wall superior to the attachment of the middle turbinate
AMICAR = amino caproic acid; MC = methyl cellulose

Treatment Sheep number	Normal saline	AMICAR +/- MC
1	6	6
2	8	6
3	6	4
4	8	6
5	6	6
6	4	4
7	4	2
8	4	4
9	4	6
10	6	6

Table 13: Total time (minutes) to achieve complete haemostasis in the lateral nasal wall inferior to the attachment of the middle turbinate
AMICAR = amino caproic acid; MC = methyl cellulose

Treatment Sheep number	Normal saline	AMICAR +/- MC
1	10	10
2	14	9
3	12	8
4	8	12
5	10	12
6	8	10
7	6	10
8	8	4
9	8	4
10	8	6

The mean time (minutes) to achieve complete haemostasis for the 4 injured sites with normal saline, AMICAR and AMICAR with methyl cellulose are given in table 12.

Table 14: Mean time (minutes) to achieve complete haemostasis
LNW = lateral nasal wall; AMICAR = amino caproic acid, MC = methyl cellulose

Treatment Site	Normal saline (n=10)	AMICAR (n=5)	AMICAR + MC (n=5)	AMICAR +/- MC (n=5)
Ethmoturbinals	4.8	4.9	3.9	4.4
Maxillary ostium	5.4	6.4	4.8	5.6
LNW (superior)	5.6	4.8	4.4	4.6
LNW (inferior)	9.2	10.4	6.8	8.75

The results of a paired two tailed Student's t test for the 4 sites, with normal saline versus AMICAR with or without methyl cellulose is given in table 12.

Table 15: Paired two tailed t test of time to achieve complete haemostasis, for each surgical site – normal saline vs AMICAR +/- MC (n=10)
LNW (superior) = lateral nasal wall superior to middle turbinate attachment
LNW (inferior) = lateral nasal wall inferior to middle turbinate attachment

Site	t	p
Ethmoturbinals	0.4	0.7
Maxillary ostium	0.48	0.64
LNW (superior)	1.41	0.19
LNW (inferior)	0.63	0.55

The results of a paired two tailed Student's t test for pooled data for all 4 sites are shown in table 16.

Table 16: Paired two tailed t test for each treatment group

MC = methyl cellulose

Treatment	t statistic	p value
Normal saline vs AMICAR	1.96	0.065
Normal saline vs AMCIAR + MC	-0.14	0.89
Normal saline vs AMICAR +/- MC	1.68	0.19

There was no significant effect on the time to achieve complete haemostasis with the use of AMICAR on any of the 4 sites examined. However there was a trend (p=0.065) towards a shorter time to achieve haemostasis with the use of AMICAR alone when data for all sites were pooled.

6. Formation of Adhesions and Synechiae

6.1 Incidence of Adhesions between ethmoturbinals and the adjacent lateral nasal wall

Table 17 below shows the incidence of adhesions between the ethmoturbinal and the adjacent lateral nasal wall in all sheep, on the normal saline treated side and the AMICAR treated side, at 28, 56, 84 and 112 days after the initial injury and treatment.

Table 17: Incidence of adhesions between the ethmoturbinals and the adjacent lateral nasal wall

- = no adhesions; + = adhesions observed

Sheep number	Normal saline treated side				AMICAR treated side			
Day from treatment	28	56	84	112	28	56	84	112
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	+
3	-	-	+	+	-	+	+	+
4	-	+	+	+	-	+	+	+
5	-	-	-	-	-	-	-	-
6	+	+	+	+	-	-	-	-
7	+	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-
10	+	+	+	+	+	+	+	+

A chi-squared test with Yates correction factor was applied to the above data, to analyse the relationship between the treatment received and the incidence of adhesions. The following table shows the chi-squared values and p values.

Figure 17 and 18 are endoscopic views of an adhesion between the ethmoturbinal and the lateral nasal wall. Note *Oestrus ovis* larvae within the nasal cavity.

Table 18. Incidence of adhesions with AMICAR and NS, chi-squared values (Yates corrected) and p values

Time from treatment	χ^2	p
28 days	0.3125	>0.25
56 days	0.2374	>0.10
84 days	3.6666	>0.05
112 days	0.208	>0.25

Figure 17: Endoscopic view of early adhesions between ethmoturbinals in the right nasal cavity. The nasal septum is to the right hand side. Note *Oestrus ovis* larva on the lateral ethmoturbinal.

Figure 18: Endoscopic view of adhesions between the ethmoturbinals, lateral nasal wall and the nasal septum in the left nasal cavity. The ridge on the left hand side surface is the remnant of the middle turbinate. Note *Oestrus ovis* larva on the nasal septum.

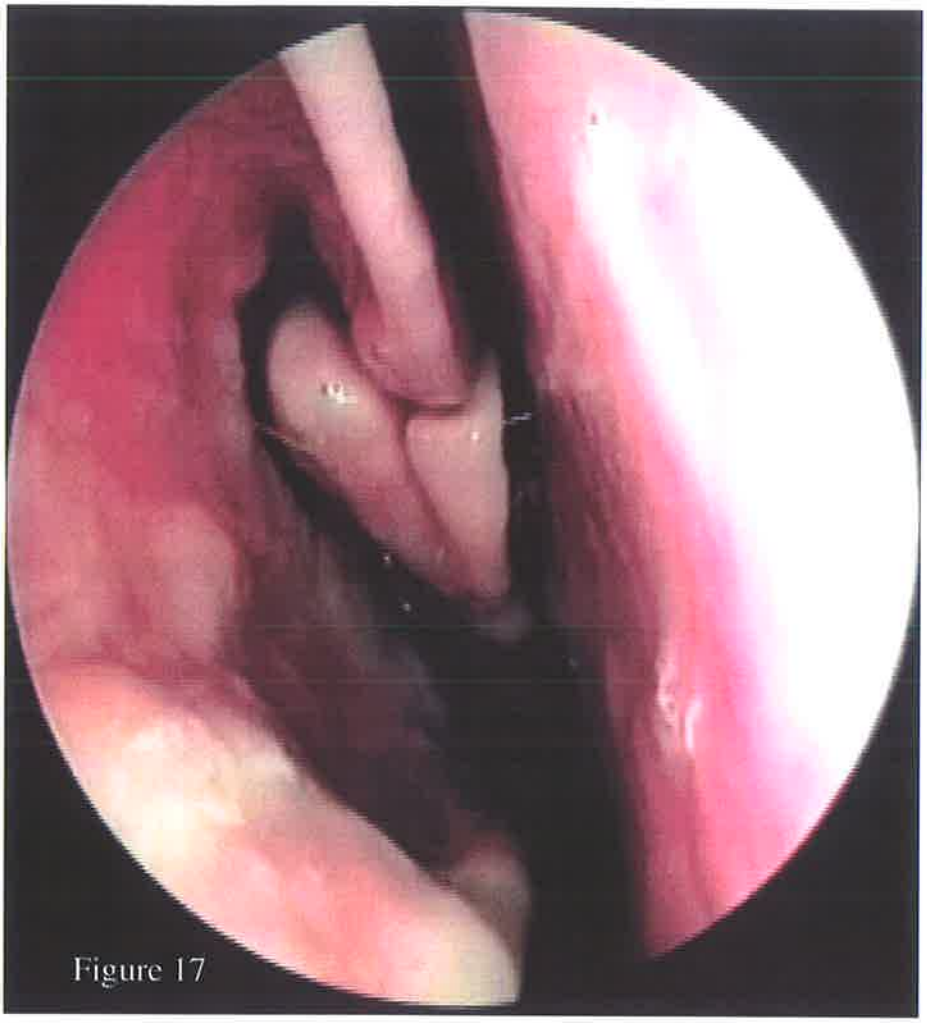


Figure 17

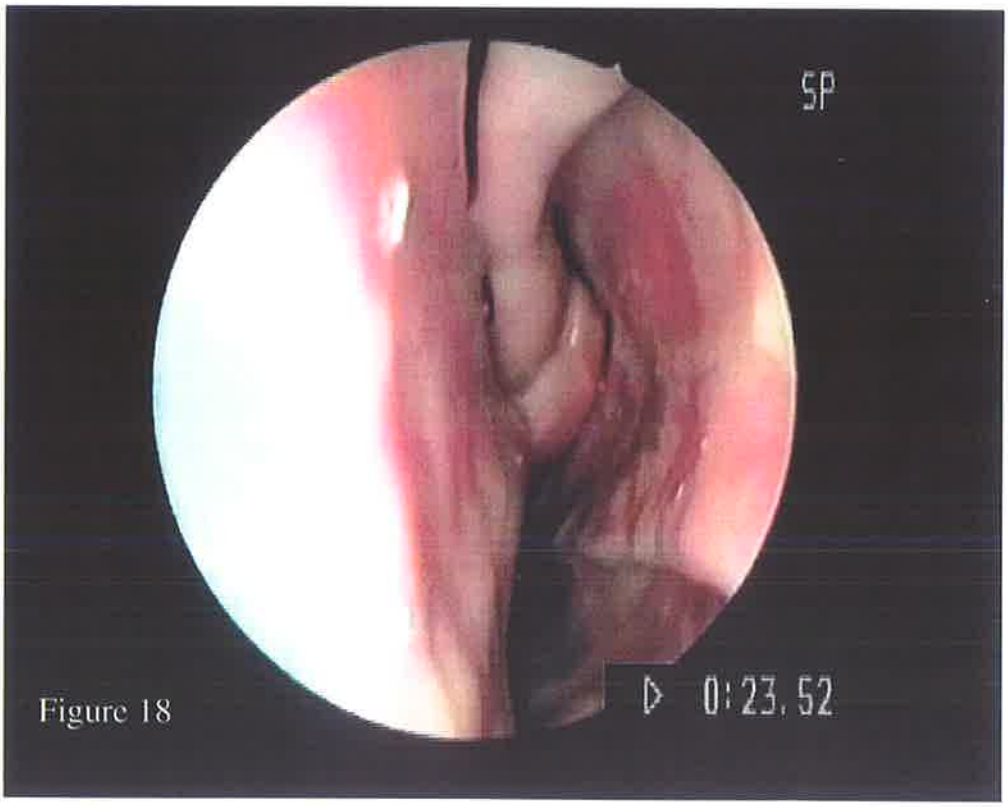


Figure 18

There was no relationship between the incidence of adhesions and the treatment received.

6.2 Incidence of Synechiae in the maxillary ostium

Table 19 below shows the incidence of synechiae in the maxillary ostium in the normal saline treated side and the AMICAR treated side in all sheep at 28, 56, 84 and 112 days after initial injury and treatment.

Table 19: Incidence of synechiae in the maxillary ostium

- = no synechiae; + = synechiae observed

Sheep number	Normal saline treated side				AMICAR treated side			
Day from treatment	28	56	84	112	28	56	84	112
1	-	-	-	+	-	-	-	-
2	-	-	-	+	-	-	-	+
3	-	-	-	-	-	-	-	+
4	-	-	-	+	-	-	-	-
5	--	-	-	-	-	-	-	+
6	+	+	+		-	-	+	
7	+	-	-		-	-	-	
8	-	-	-		-	-	-	
9	-	-	-		-	-	+	
10	+	+			--	-		

The above data were analysed using chi-squared values with Yates correction factor. No relationship was found between the incidence of synechiae formation and the treatment received.

Table 20: Incidence of synechiae with AMICAR and normal saline, chi-squared values (Yates corrected) and p values

Time from treatment	χ^2	p
28 days	1.575	>0.10
56 days	0.811	>0.25
84 days	0.1875	>0.25
112 days	0	>0.25

7. Re-epithelialisation

The percentage area re-epithelialised on the lateral nasal wall was calculated using the biopsy specimens. The following table gives the summary of descriptive statistics for re-epithelialisation in all sheep.

Table 21. Summary statistics of percentage area of epithelialisation observed under the light microscope for biopsies at each time interval following surgery for normal saline and AMICAR+/- MC (AM) treated sides

Day post surgery	28	28	56	56	84	84	112	112
Treatment	NS	AM	NS	AM	NS	AM	NS	AM
Mean % area of epithelialisation	79.68	77.09	96.88	100	97	100	98	100
95% CI	66.47-92.87	45.6-98.58	89.49-100	100	91.19-100	100	93.48-100	100
SEM	5.73	9.09	3.13	0	2.49	0	2	0
SD	17.18	25.71	8.84	0	7.01	0	6.02	0

The percentage re-epithelialisation for each treatment group (normal saline and AMICAR+/-HPMC) at each time interval is shown in figure 19.

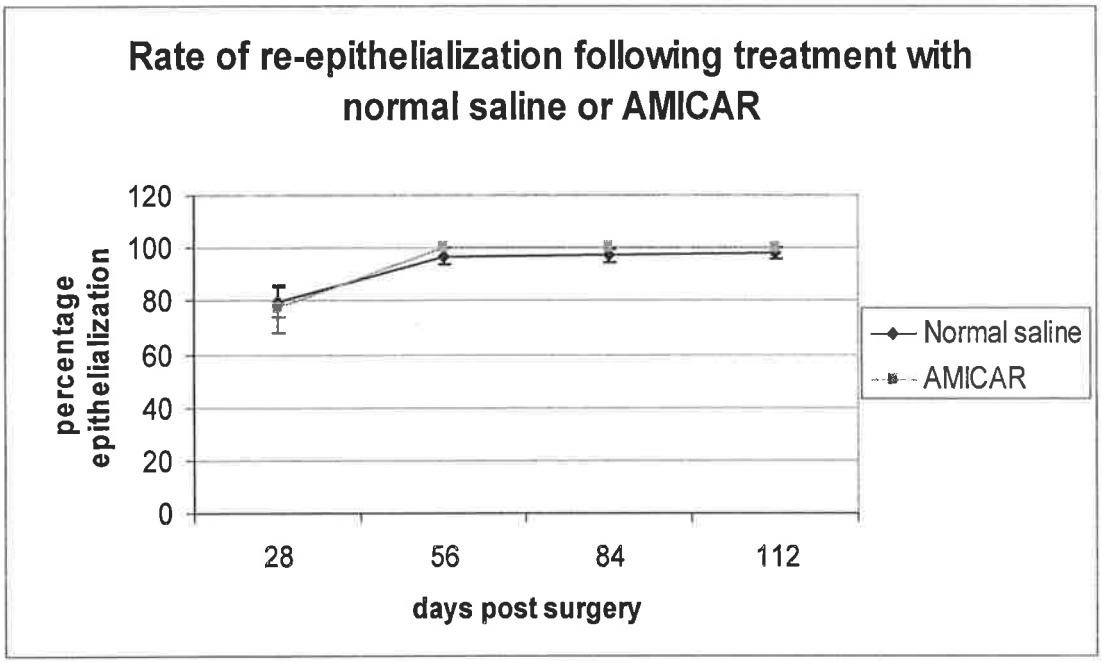


Figure 19: Percentage area epithelialisation versus the number of days post surgery for normal saline treated and AMICAR+/- methyl cellulose treated sides in all sheep

Figure 20 shows an area of re-epithelialisation in an AMICAR treated sheep, at day 56 biopsy and figure 21 is the same sheep at day 84 at a magnification of 20 under a light microscope following staining with haematoxylin and eosin.

The percentage data were transformed to arcsine and a paired two tailed t test was applied. The p values for epithelialisation in normal saline treated vs AMICAR treated sides at day 28, day 56, day 84 and day 112 were 0.51, 0.37, 0.76 and 0.34 respectively. There was no significant difference in the rate of re-epithelialisation at any time point when normal saline was compared with AMICAR.

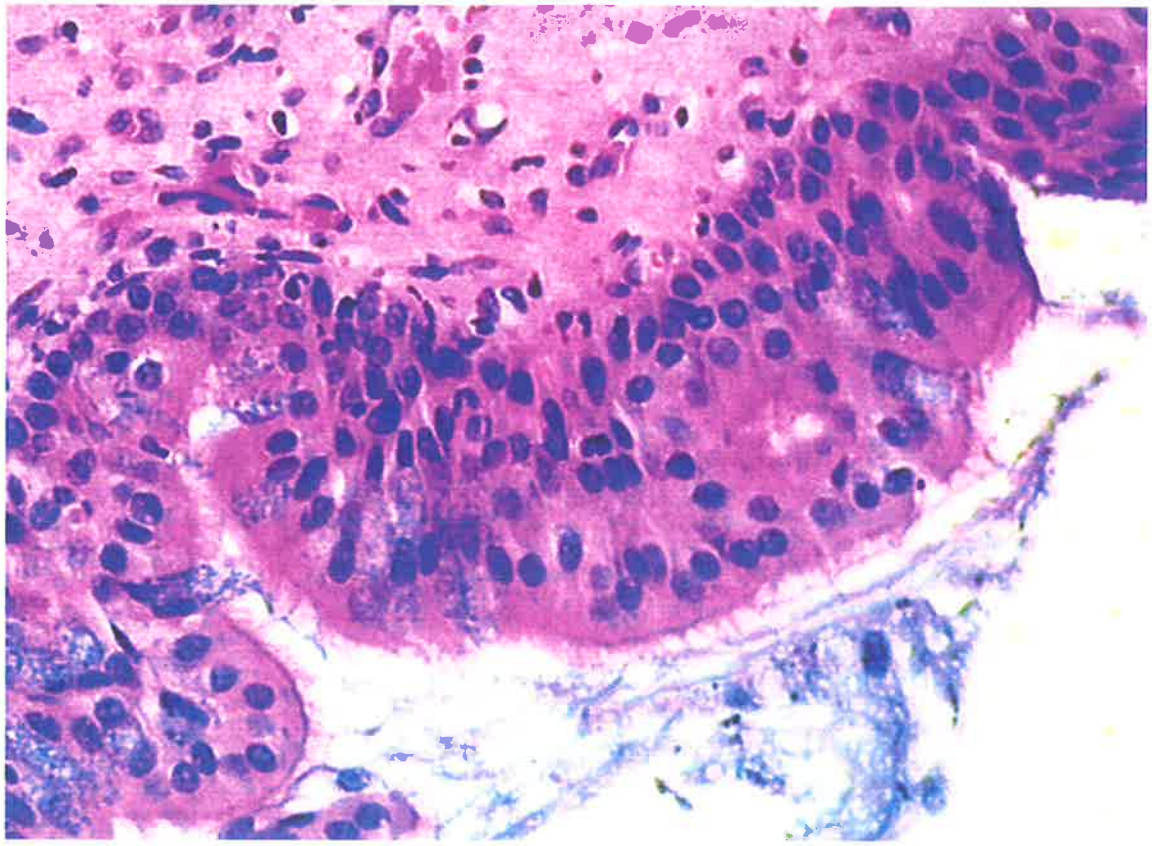


Figure 20: Light micrograph of nasal epithelium on day 56 following treatment with AMICAR, Haematoxylin and eosin stain, x20.

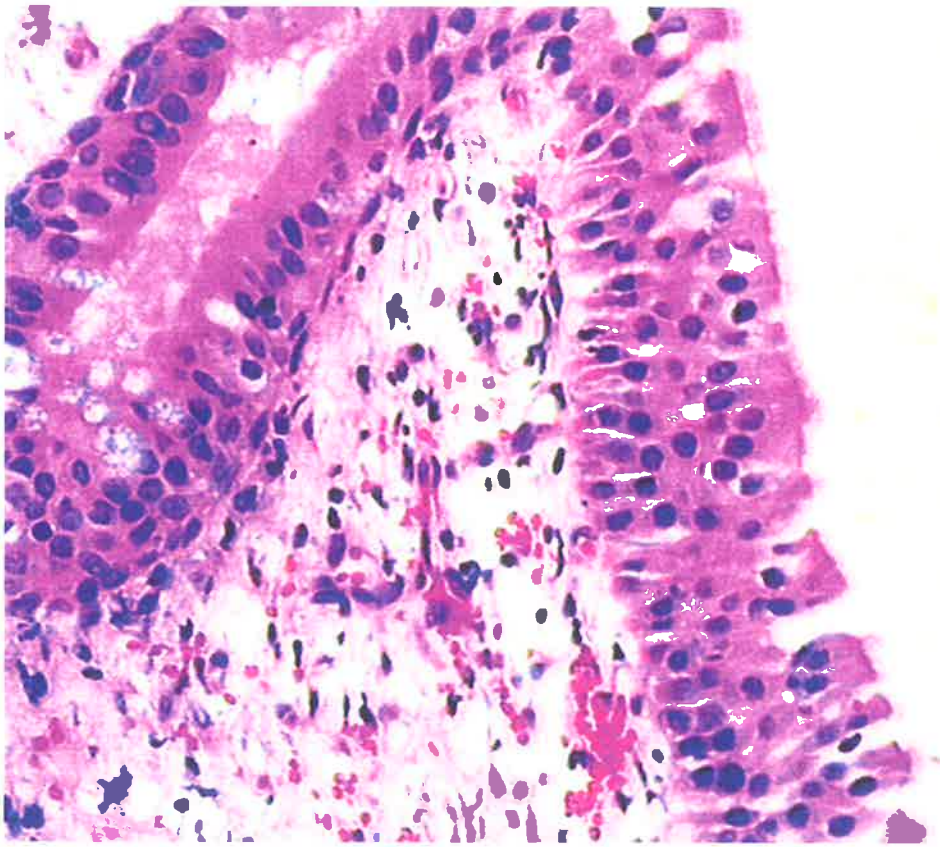


Figure 21: Light micrograph of nasal epithelium following treatment with AMICAR, H&E stain, x20

Table 22. The comparison of the rate of epithelialisation between normal saline treated side and AMICAR treated side in all sheep

Treatment/ biopsy (days)	NS 28	AMICAR 28	NS 56	AMICAR	NS 84	AMICAR 84	NS 112	AMICAR 112
Mean	69.41	67.13	84	90	85.24	90	87.34	90
Variance	317.88	474.23	180	0	93.88	o	70.54	0
n	8	8	5	5	8	8	10	10
df	7		4		7		9	
t	2.36		2.78		2.36		2.26	
p	0.51		0.37		0.75		0.34	

Table 23. Summary statistics of the percentage area of epithelialisation at each time interval following surgery in sheep treated with AMICAR and MC (AM+MC)on the same side Sheep 6-10)

Day post surgery	28	28	56	56	84	84	112	112
Treatment	NS	AM + MC	NS	AM + MC	NS	AM + MC	NS	AM + MC
Mean % area of epithelialisation	88.05	82.64	100	100	99	100	100	100
95% CI	50.05-76.07	27.41-100	100	100	95.82-100	100	100	100
SEM	5.73	9.09	3.13	0	2.49	0	2	0
SD	17.18	25.71	8.84	0	7.01	0	6.02	0

When normal saline treated sides were compared with AMICAR combined with MC treated sides using a two tailed paired t – test, the p values at day 28, day 56, day 84 and day 112 were 0.39, >05, 0.32 and >0.5 respectively. Thus there was no significant difference in the rate of epithelialisation between the normal saline treated sides and the AMICAR combined with HPMC treated sides.

Table 24. Comparison of the rate of epithelialisation between sheep treated with AMICAR alone (AM) vs AMICAR combined with methyl cellulose (AM+MC).

Treatment/ biopsy (days)	AM 28	AM+MC 28	AM 56	AM+MC 56	AM 84	AM+MC 84	AM 112	AM+MC 112
Mean	58.38	75.88	90	90	90	90	90	90
Variance	104.94	797.49	0	0	0	0	0	0
n	4	4	2	2	4	84	5	5
df	3		1		3		4	
t	3.18		12.71		3.18		NA	
p	0.64		0.5		1		NA	

There was no significant difference in the rate of epithelialisation between sheep treated with AMICAR alone and those treated with AMICAR and methyl cellulose.

8. Epithelial height

As shown in figure 22 there was a general trend for the epithelial height to increase with time. The preoperative epithelial height is similar to that seen in day 28 and 56 but from day 84 onwards the epithelial height appears to increase.

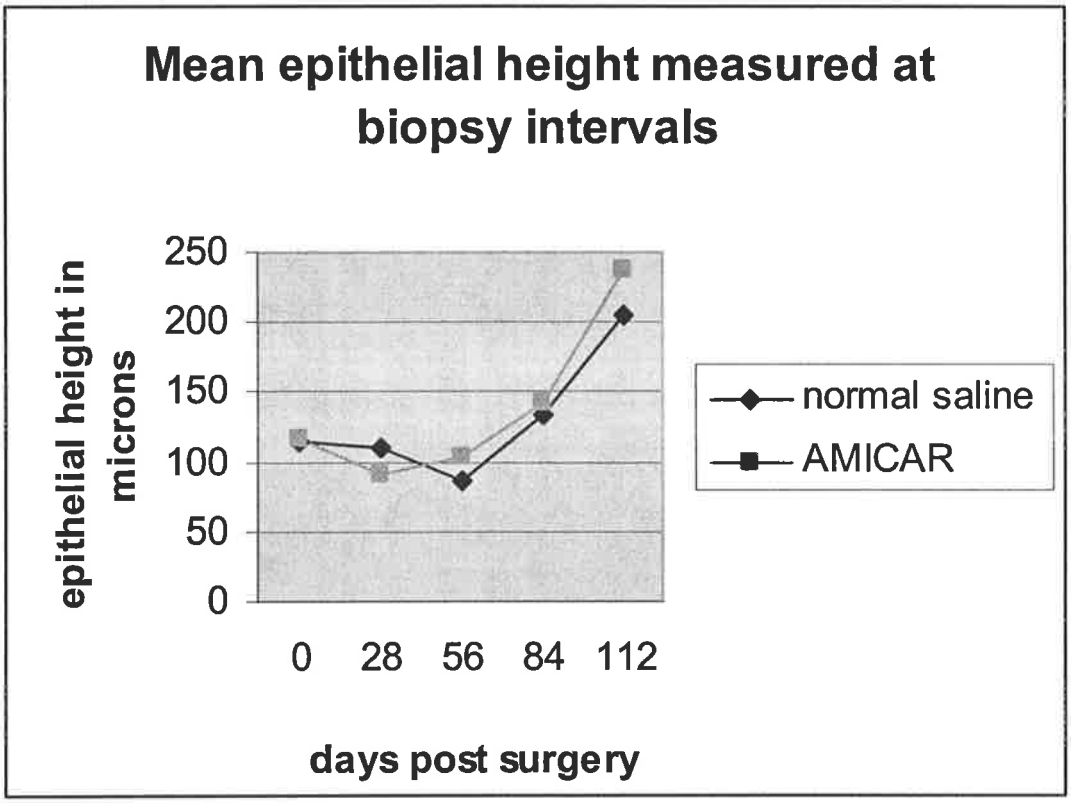


Figure 22. Epithelial height (microns) measured at each biopsy interval.

There was no significant difference in the epithelial height between day 0 and day 28 (p=0.73), day 28 and day 56 (p=0.23), day 56 and day 84 (p=0.15) in the saline treated side. The epithelial height was significantly higher at day 112 compared to day 84 biopsy in the saline treated side (p=0.02).

Similarly there was no significant difference in the epithelial height between day 0 and day 28 (p=0.29), day 28 and day 56 (p=0.99) and day 56 and day 84 (p=0.82) in the AMICAR treated side. There was a significant increase in the epithelial height at day 112 compared to day 84 in the AMICAR treated side (p=0.015).

When the epithelial height of the saline treated side was compared with that of AMICAR treated side using a two tailed t test for means, there was no significant difference at any time interval. (p=0.51, 0.07, 0.11, 0.30 and 0.77 at day 0, 28, 56, 84 and 112 respectively).

9. Re-ciliation

9.1 Percentage area of epithelium with mature cilia

Table 25. Descriptive statistics of reciliation with mature cilia at each biopsy interval following post operative treatment with normal saline or AMICAR+/-MC (AM)

NS/AM biopsy	NS 28	AM 28	NS 56	AM 56	NS 84	AM 84	NS 112	AM 112
Mean % area of reciliation	6	5.6	20.1	36.3	71.11	42	51.11	56.5
95% CI	5.29-16.29	6.16-16.84	5.46-34.74	16.12-46.48	47.59-94.63	15.9-68.1	18.17-84.05	34.01-78.99
SEM	4.99	4.97	6.43	8.92	10.19	11.04	14.28	9.95
SD	15.77	15.71	19.05	28.21	30.59	31.22	42.85	31.45

The mean percentage area of mature cilia observed in the normal saline treated side and AMICAR with/without MC are given in the following graph for each time interval following surgery (n=10).

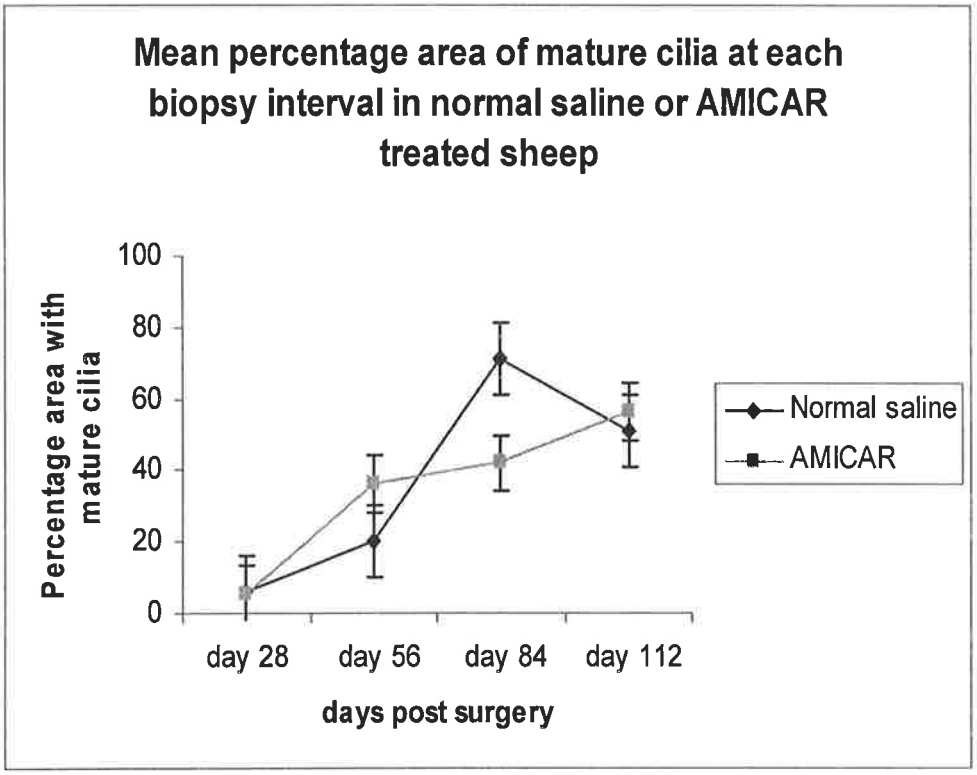


Figure 23. Mean percentage area of mature cilia at each biopsy interval following surgery in normal saline treated and AMICAR +/- MC treated sides.

The percentage data were transformed into arcsine values and a paired two tailed t test was applied to them. The p value for normal saline vs AMICAR+/-MC at day 28, 56, 84 and 112 were 0.32, 0.23, 0.04 and 0.53 respectively. Reciliation with mature cilia at day 84 was significantly higher in the normal saline treated side when compared with the AMICAR treated side. There was no significant difference in the rate of ciliation with mature cilia between the saline and AMICAR treated sides at the other 3 time intervals.

Table 26. The summary statistics for percentage area of reciliation with mature cilia following arsine transformation for all sheep (NS = normal saline; AM = AMICAR)

NS/AM biopsy	NS 28	AM 28	NS 56	AM 56	NS 84	AM 84	NS 112	AM 112
Mean	3.19	5.95	22.81	36.49	61.26	42.77	44.43	54.07
Variance	46.56	209.1	275.24	528.54	643.51	449.73	1058.3	373.19
n	10	10	9	9	8	8	9	9
df	9		8		7		8	
t	2.26		2.31		2.36		2.31	
p	0.32		0.23		0.04		0.53	

The comparison of the rate of ciliary maturation as measured by the percentage area of epithelium covered with morphologically mature cilia shows that there was a significantly higher rate of ciliary maturation on the side treated with normal saline on day 84 ($p=0.04$). No significant differences were observed on the other biopsies.

Table 27. The percentage area of epithelium containing morphologically mature cilia in sheep treated with AMICAR alone (AM) vs sheep treated with AMICAR and methyl cellulose (AM+MC).

Treatment/ biopsy (days)	AM 28	AM+ MC 28	AM 56	AM+ MC	AM 84	AM+ MC 84	AM 112	AM+ MC 112
Mean	2.91	9	44.04	30.65	35.19	50.35	61.6	45.86
Variance	42.28	405	828.49	132.71	784.54	111.68	457.43	369.71
n	5	5	5	5	4	4	4	4
df	4		4		3		3	
t	2.78		2.78		3.18		3.18	
p	0.37		0.35		0.48		0.92	

When the rate of mature ciliation was compared between the sheep treated with AMICAR alone and AMICAR combined with MC using a paired two tailed t test the p values at day 28, 56, 84 and 112 were 0.37, 0.36, 0.48 and 0.92 respectively. Thus the addition of mucoadhesive MC did not affect the rate of reciliation or the maturation of cilia significantly.

6.2 Percentage area of epithelium with immature cilia

The percentage area covered with morphologically immature cilia (short) was calculated using scanning electron microscopy and image analysis software. The percentage area for each biopsy was a mean of 3 microscopic fields observed. The mean value was obtained for each treatment group at each biopsy interval. Table 28 gives the percentage data and figure 24 A is an electron micrograph of an area of re-epithelialisation with immature cilia and figure 24 B an area of re-epithelialisation with mature cilia at a magnification of 3500. Figure 25 shows the percentage area of reciliation with immature cilia graphically.

Table 28. Summary statistics for the mean percentage area with immature cilia in all sheep (NS = normal saline; AM = AMICAR).

NS/AM biopsy	NS 28	AM 28	NS 56	AM 56	NS 84	AM 84	NS 112	AM 112
Mean % area of reciliation	33	44.5	43.75	39	25.22	33.75	43.44	43.5
95% CI	20.80	29.89	24.45	19.50	22.21	30.62	30.26	23.73
SEM	9.19	13.21	10.34	6.62	9.63	12.94	13.12	10.48
SD	29.07	41.79	29.25	27.26	28.89	36.62	39.37	33.17

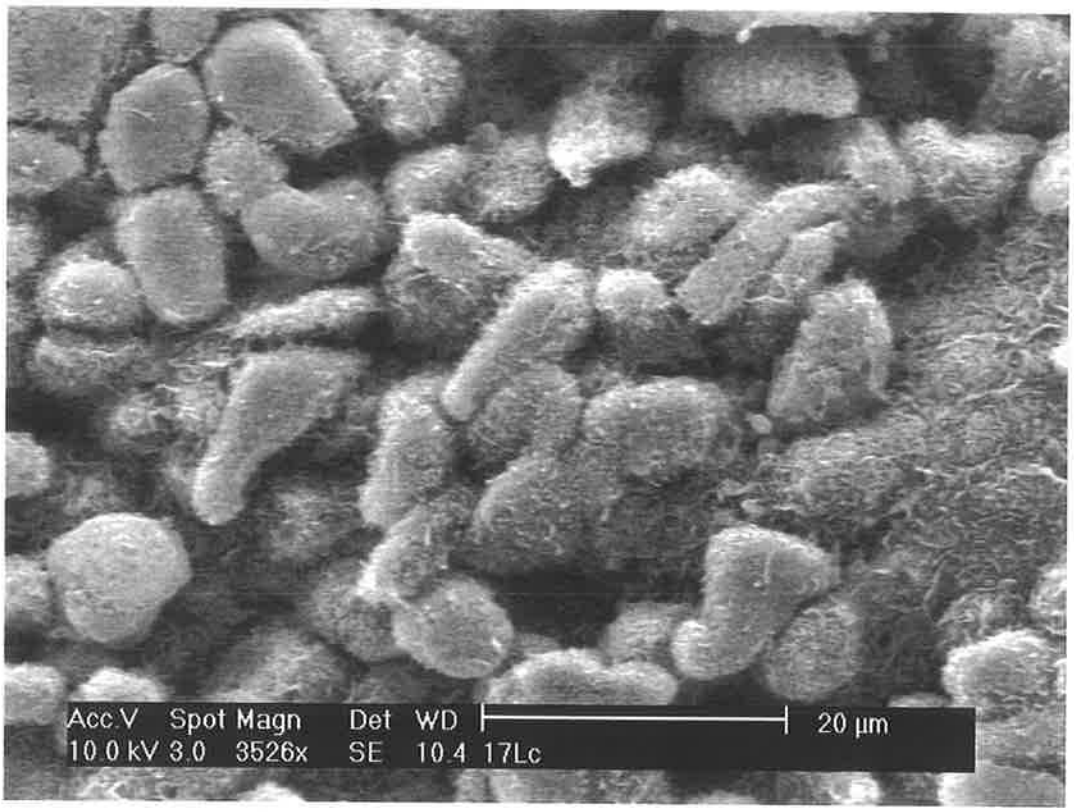


Figure24A: scanning electron micrograph of a biopsy of the lateral nasal wall at day 28, showing immature cilia at a magnification of 3500.

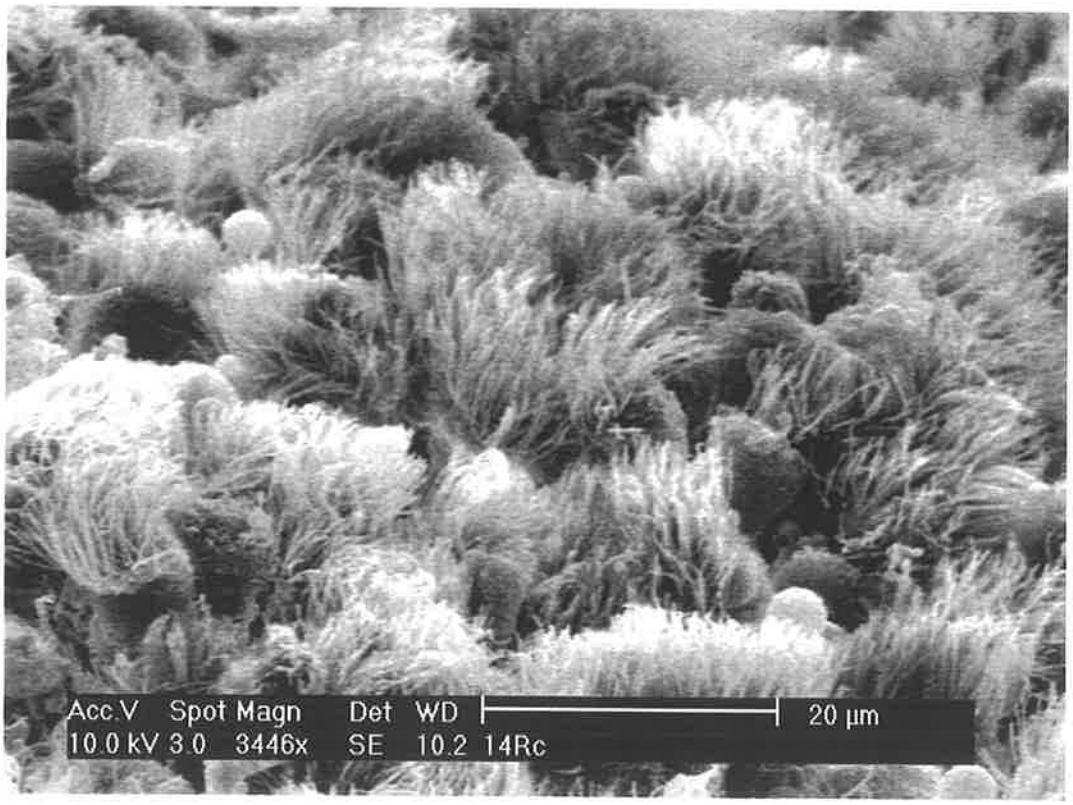


Figure24B: Scanning electron micrograph of mature cilia at a magnification of 3500.

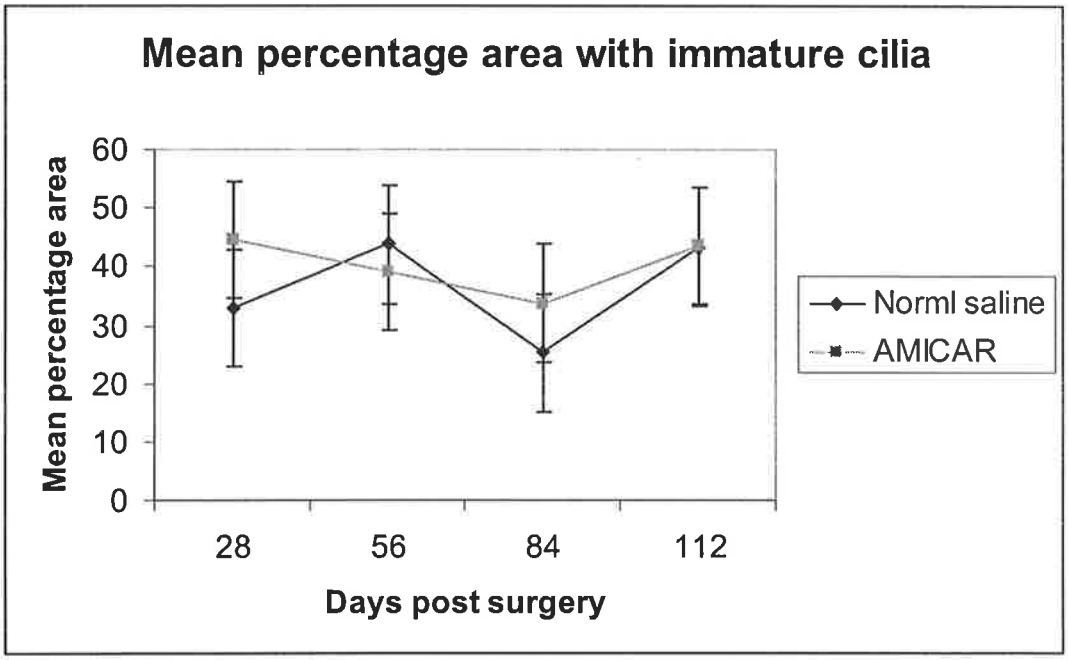


Figure 25. The mean percentage area with immature cilia at each biopsy interval in all sheep, treated with normal saline and AMICAR (sheep 1 to 5) and AMICAR with methyl cellulose (sheep 6 to 10).

The percentage data were then transformed using an arcsine table. A two tailed t test for sample means was applied to the transformed data. The table below summarises the data.

Table 29. Summary statistics for mean percentage area with immature cilia for all sheep (arsine transformed data) (NS = normal saline; AM = AMICAR).

NS/AM	NS	AM	NS	AM	NS	AM	NS	AM
biopsy	28	28	56	56	84	84	112	112
Mean	30.56	38.68	40.39	40.39	22.5	33.29	37.72	32.74
Variance	578.49	1346.05	490.2	414.35	675.72	925.34	863.35	267.35
n	10	10	8	8	8	8	9	9
df	9		7		7		8	
t	2.26		2.36		2.36		2.30	
p	0.35		0.73		0.27		0.67	

7.3 Mean percentage area of total ciliation

The area of total ciliation was calculated by adding the mature and immature ciliated areas for each sheep. The mean area of total ciliation was calculated for each treatment group, at each biopsy interval. The percentage data are shown in the table below.

Table 30. Descriptive statistics for the mean percentage area of total reciliation (mature and immature cilia) in all sheep (NS = normal saline; AM = AMICAR).

NS/AM biopsy	NS 28	AM 28	NS 56	AM 56	NS 84	AM 84	NS 112	AM 112
Mean % area of reciliation	34.54	50.15	59.78	70.24	66.67	93.33	94.44	98
95% CI	21.96	31.92	28.83	18.59	17.19	10.87	8.69	4.52
SEM	9.72	14.11	12.5	8.22	7.45	4.71	3.77	2
SD	30.73	44.62	37.51	25.98	22.36	14.14	11.30	6.32

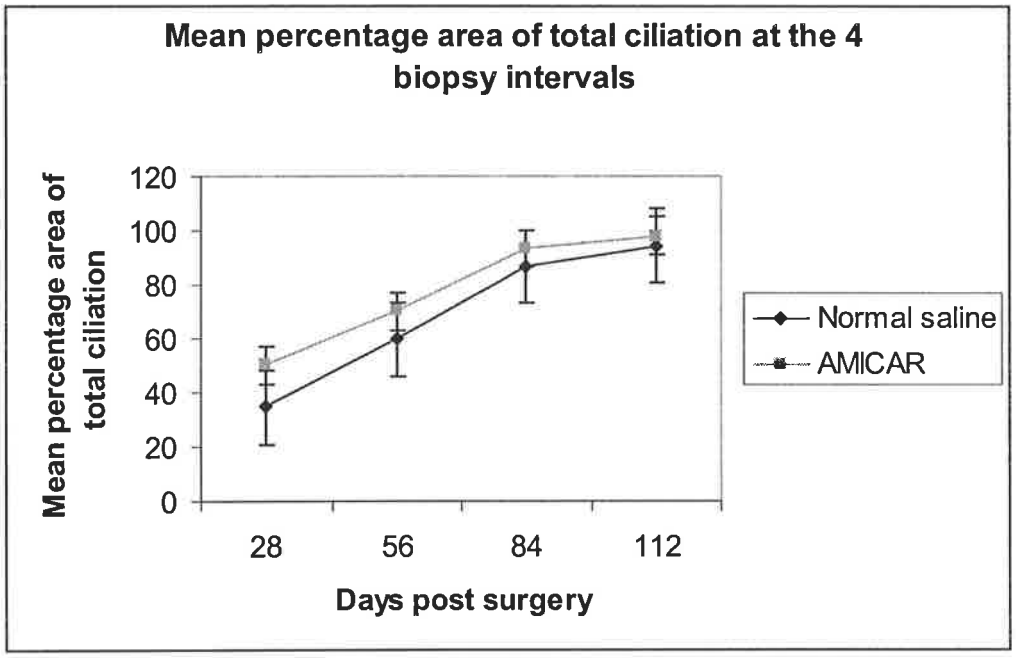


Figure 26. The mean percentage area of reciliation with mature and immature cilia in all sheep treated with normal saline and with AMICAR or AMICAR with methyl cellulose at the 4 biopsy intervals.

The percentage data for all sheep treated with normal saline and AMICAR+/- MC were then transformed using an arcsine table. A two tailed t test for sample means was applied to the transformed data. The table below summarises the data.

Table 31. Summary statistics of arsine transformed data for the mean percentage area of total ciliation (mature and immature) for all sheep NS = normal saline; AM = AMICAR

NS/AM	NS	AM	NS	AM	NS	AM	NS	AM
biopsy	28	28	56	56	84	84	112	112
Mean	33.74	44.66	51.1	62.01	77.05	82.69	83.36	87.16
Variance	701.82	1491.85	840.26	537.26	414.06	220.44	176.42	72.59
n	10	10	9	9	9	9	9	9
df	9		8		8		8	
t	2.26		2.31		2.30		2.31	
p	0.17		0.22		0.58		0.25	

The influence of AMICAR without methyl cellulose was assessed by comparing the normal saline treated side with the side treated with AMICAR alone (in sheep number 6 to 10). A two tailed t test for sample means was applied to arcsine transformed data. The table below shows the summary statistics.

Table 32. Summary statistics of arcsine transformed data for mean percentage area with total ciliation (mature and immature) for sheep treated with AMICAR alone (AM).

Treatment/ biopsy (days)	NS 28	AM 28	NS 56	AM 56	NS 84	AM 84	NS 112	AM 112
Mean	17.18	32.31	49.91	67.68	83.36	73.56	75.06	83.61
Variance	306.86	1580.73	1539.14	973.68	176.36	387.45	305.07	163.33
n	5	5	5	5	4	4	4	4
df	4		4		3		3	
t	2.78		2.78		3.18		3.18	
p	0.20		0.46		0.55		0.28	

The effects of methyl cellulose in combination with AMICAR was assessed by comparing the data for sheep treated with AMICAR alone with those treated with AMICAR and methyl cellulose using a two tailed t test for sample means.

Table 33. Comparison of total ciliation in the nasal mucosa of sheep treated with AMICAR (AM) alone vs sheep treated with AMICAR and methyl cellulose (AM+MC).

Treatment/ biopsy (days)	AM 28	AM+ MC 28	AM 56	AM+ MC 56	AM 84	AM+ MC 84	AM 112	AM+ MC 112
Mean	32.31	57	67.68	56.29	73.56	62.02	84.89	90
Variance	1580.7	1395	973.68	11.19	387.45	127.58	130.66	0
n	5	5	5	5	4	4	5	5
df	4		4		3		4	
t	2.78		2.78		3.18		2.78	
p	0.25		0.39		0.13		0.37	

No significant effect on total ciliation was seen by combining AMICAR with methyl cellulose.

DISCUSSION

1. *Oestrus ovis* infestation and resultant eosinophilic sinusitis

Oestrus ovis is a common parasite in sheep and goats. Its pathological effects are often underestimated. Experimental infestation with the parasite has uncovered detailed information about the life cycle of the parasite as well as the pathophysiological responses of the host during each of the life stages of the larvae. Within the nasal cavity and the sinuses, the epithelium shows hyperplasia and metaplasia suggesting a highly regenerative process (Tabouret *et al.,* 2003b).

In the present study, a sheep eosinophilic sinusitis model was used. The epithelial eosinophil numbers as well as secreted eosinophils in the mucus were higher in the infested sheep compared to those treated with ivermectin. In addition, the sheep infested with the parasite also showed other features of inflammation and regeneration such as high grade inflammation within the lamina propria, rounded nuclei within the epithelial cells and some dissociation between epithelial cells. These observations were also made by Hoste *et al* in 2001.

The magnitude of the cellular immune response, measured as the number of eosinophils, mast cells and globular leucocytes were found to be correlated to the larval burden albeit with a high variance of cell numbers between sheep (Nguyen *et al.,* 1999). In the present study the larval burden in each sheep was not quantified, but there was a high variance of eosinophil numbers found between sheep which may reflect variable larval burden. In addition as the cells were found in aggregates there was a high variance between different biopsy sections of the same sheep.

In order for the *O ovis* infested sheep to be used as a sheep model of eosinophilic sinusitis, secreted eosinophilia can be used as a marker of the disease. The number of eosinophils seen per high power field to confirm or diagnose eosinophilic sinusitis is defined as the average number of eosinophils per high power field in control animals plus 2 standard deviations. This allows for the variation of eosinophil numbers in normal or control animals and includes 98% of the population. Therefore the cut off

number of eosinophils required to make the diagnosis of eosinophilic sinusitis is 0.49 eosinophils per high power field, or at least 1 eosinophil per 2 high power fields. This information is vitally important for further use of the sheep model of eosinophilic sinusitis. It allows the researcher to confirm that the sheep to be involved in a particular study to have eosinophilic chronic sinusitis using a simple nasal swab assessment. One of the criticisms in the past has been the inability of the researcher to prove the presence of eosinophilic chronic rhinosinusitis in sheep where the larvae were not visible.

All sheep in the present study were naturally infected with *Oestrus ovis* and reared outdoors, with exposure to flies. A seasonal variation in fly numbers is well known and the larvae are most active in spring. The mucosal injuries in all sheep were not performed during the same time of the year, but rather were spaced throughout the year. The larval burden may have varied in sheep depending on the month of the year and therefore the eosinophil counts in the initial biopsy may have varied as a reflection of this. Furthermore there may have been implications on the rate of re-epithelialisation, re-ciliation and ciliary maturation from the larval burden during the period of repair. Thus variance within groups for each of these observations may have been less if all sheep were operated on in the same season.

2. Haemostatic effects of AMICAR

Endoscopic sinus surgery is a common and standard procedure for chronic sinusitis. This procedure is commonly associated with bleeding during and following surgery. Bleeding during and after surgery is minimised by preoperative and intraoperative interventions. Preoperative interventions such as oral steroids in patients with nasal polyps can reduce vascularity of polyps and reduce bleeding (Orlandi and Lanza 2004). Intraoperative measures include management of the patient's pulse rate and cardiac output (Nair *et al.,* 2004), decreasing vasodilation by utilising total intravenous anaesthesia (TIVA) (Wormald *et al.,* 2005a), topical anaesthesia with vasoconstrictors such as cocaine and adrenaline and injected lignocaine and adrenaline. Local anaesthetic blocks such as the pterygopalatine fossa block

(Wormald *et al.,* 2005b) is an example. In order to control post operative bleeding a variety of nasal packs have been used after ESS. In addition packing is thought to prevent adhesion formation (Brennan 1996). However nasal packing is not without its own complications. Patients report discomfort during removal of packs (Garth and Brightwell 1994) and its associated trauma is evident with bleeding after nasal pack removal being very common and unpleasant to patients. In fact most patients state that the nasal packing and its subsequent removal were the most unpleasant part of the surgical experience. In addition the trauma associated with the nasal pack removal can interfere with the healing of the mucosa, as tissue that is under pressure and in contact with the mucosa is damaged as the pack is withdrawn. Nasal packing has been shown to result in the loss of ciliated epithelium (Shaw *et al.,* 2000).

In recent years a number of dissolved nasal packs have been developed in an attempt to overcome some of these problems. The most widely used dissolvable pack is hyaluronic acid, which appears to facilitate cell migration, matrix formation and healing. It is an important component in fetal wound healing where it may play a role in allowing fetal wounds to heal without scar formation (Weigel *et al.,*1986). In sheep without eosinophilic chronic rhinosinusitis this esterified hyaluronic acid nasal pack (Merogel ® Medtronic Xomed FI USA) improved the speed of re- epithelialisation when applied to nasal mucosal wounds (McIntosh *et al.,* 2002). However this improvement appeared to be lost when this pack was used in sheep with eosinophilic chronic rhinosinusitis (Rajapakse *et al.*, 2005b). While hyaluronic acid was intended to improve healing after surgery in the nose it still did not provide a solution for ongoing bleeding after ESS. Topical haemostatic agents have been developed in an attempt to achieve haemostasis and to try to improve healing after ESS (Chandra *et al.*, 2003). Floseal ® was the first of these agents developed and consists of bovine gelatin mixed with thrombin. In addition platelet-rich gels and tissue adhesives with fibrin have been suggested. Fibrin sealants are the most effective tissue adhesive currently available (Jackson 2001). They contain human fibrinogen, human thrombin, factor XIII and bovine aprotinin. These mimic the final stages of physiological coagulation resulting in a stable clot. They are widely used in cardiovascular, orthopaedic, and neurosurgery (Spotnitz 2001). The effectiveness of these products on haemostasis and their effect on re-epithelialisation and adhesion formation are still to be determined. In addition the effect of the blood clot on the healing of a mucosal

surface has not been elucidated. FloSeal, an absorbable haemostatic agent in the form of bovine collagen with thrombin has been shown to significantly increase the rate of granulation tissue formation and adhesions compared to thrombin-soaked foam (Chandra *et al.*, 2003). In other studies it was shown to have no effect on haemostasis compared to hyaluronic acid packs (Baumann and Caversaccio 2003; Chandra *et al.*, 2003) but was associated with a high degree of patient comfort (Chandra *et al.*, 2003). Yet other studies report adequate control of bleeding with FloSeal ® in ESS (Gall *et al.,* 2002). Used in adenoidectomy in children however, FloSeal ® was shown to significantly reduce the time to achieve haemostasis and reduced the length of surgery (Mathiasen and Cruz 2004). Autologous fibrin tissue adhesive was found to provide haemostasis as well as to increase patient satisfaction (Gleich *et al.,* 1995). Despite some initial good results FloSeal® has lost appeal due to a significant increase in post operative adhesions seen in patients in whom it was used. Therefore a suitable agent for use after ESS that provides haemostasis, improves healing and reduces the rate of adhesion formation remains elusive.

One of the aims of this thesis was to evaluate AMICAR as a possible haemostatic agent that could be sprayed on the operated site at the end of surgery thereby removing the need for nasal packing. In this study we found that AMICAR when applied to the surgical wound after surgery resulted in a significant reduction in the grade of bleeding seen on the lateral nasal wall compared to the side which received the normal saline spray. Therefore the use of AMICAR alone as a hydrophilic agent may reduce the degree of bleeding and offer clear visibility during surgery. AMICAR has never been used in endoscopic sinus surgery in humans.

During this study it was noted that the AMICAR was easily washed away by the bleeding and therefore seemed to have a limited time of contact with the wounded area. In an attempt to prolong the contact time and thereby improve haemostasis AMICAR was mixed with mucoadhesive methyl cellulose. The desired effect was for AMICAR and methyl cellulose to bind at a molecular level and for the methyl cellulose to bind to the injured mucosa. However, from the pilot data presented in this study, AMICAR in combination with methyl cellulose was no different from normal saline in reducing the amount of bleeding. In the region of the maxillary ostium the normal saline performed better than the AMICAR mixed with methyl cellulose.

The method of mixing used may not have been effective, as chemical bonding between the two molecules is required in order for the hydrophobic methyl cellulose to act as a vehicle of hydrophilic AMICAR. Secondly, the dissociated methyl cellulose may have bound to the mucosal surface and prevented AMICAR from coming to contact with the injured surface. Another possibility is that once the mucosa is injured a hydrophilic or aqueous substance may bind to the surface better than a hydrophobic substance, which may bind to the intact epithelium.

Furthermore, a concentration of AMICAR of 10 to 32.5μg/ml is required to achieve antifibrinolytic effects (Ablondi *et al.*, 1959). Although these concentrations have been achieved within the aqueous humor with topical application over the eye, no studies have been done to calculate the tissue concentrations resulting from local application over mucus membranes. In order for the molecule to remain on the surface for a sufficient time in a clinically relevant concentration, a tissue or clot permeation enhancer may be required as its vehicle. On the corneal surface Carboxypolymethylene was shown to be the most effective vehicle which resulted in a duration of action of 6 hours, when applied with AMICAR (Ehlers *et al.,* 1990).

The use of AMICAR with or without the mucoadhesive agent did not significantly affect the time to achieve complete haemostasis. However there was a trend towards a shorter time to achieve haemostasis when AMICAR was used and when data for all sites were pooled. In some previous studies done using other haemostatic agents within the human nasal cavity following ESS, the time to achieve total haemostasis was not measured. Instead, the need for additional packing was used as a measure of effective haemostasis (Chandra *et al.,* 2003) and general observation after the application of the substance or on post operative visits were used to determine the extent of bleeding (Gleich *et al.*, 1995). Gall et al (2002) used a 5 point visual analogue scale to determine the severity of bleeding in the operative site prior to and after the application of FloSeal, and the time to complete cessation of bleeding.

Furthermore, in the current study the control side received equal volumes of normal saline spray on the injured surface of the nasal cavity. This provided some degree of irrigation and may have aided in haemostasis. If the control side was left untreated and did not receive saline spray there may have been a more significant difference

between the treatment side and control side in terms of degree of haemostasis and time to achieve total haemostasis.

There was a good correlation between the grades of surgical field bleeding of the superior and inferior lateral nasal wall showing consistency in the visual analog scale used in the present study. The degree of bleeding did not correlate to the extent of eosinophilia, either in the epithelium or the mucous. The eosinophils were seen within the epithelium and in the secreted mucus as a local response to the presence of *Oestrus ovis* larvae with resultant inflammation of the surface epithelium. While the degree of eosinophilia reflects the degree of local inflammation it did not have any effect on the grade of bleeding of the surgical field when a full thickness mucosal injury was created. This was surprising as one would think that the higher the degree of inflammation, the more likely the wound is to bleed. However, this result may suggest that the extent of wounding was such that any increase in bleeding was not of sufficient quantity to be detectable using the methods in this study. In addition *Oestrus ovis* infestation may cause a local inflammatory reaction which does not affect the underlying deep layers of the mucosa (Nguyen *et al.,* 1999). It may be that if the injuries were made in the ethmoidal region, a relationship could have been seen between the level of eosinophilia and the degree of bleeding as a more prominent local inflammatory reaction has been noted in the ethmoidal and sinus mucosa than the lateral nasal wall of the sheep (Tabouret *et al.,* 2003b). This is thought to be due to the highly antigenic second and third instar larval development within the ethmoidal region.

3. Adhesion and synechiae formation

In intra abdominal surgery it has been shown that the use of viscous solutions and hydrophilic polymers may minimise the formation of postoperative adhesions (Goldberg *et al.*, 1980). One theory suggests that adhesions are formed as a result of failure to enzymatically dissolve the fibrinous exudate (Buckman *et al.,* 1976) because suppression of fibrinolysis has been shown to lead to increased adhesions (Raftery 1981). It was thought that AMICAR may have an effect on adhesion formation as its mode of action is to prevent fibrinolysis.

Other dissolvable nasal packs (Merogel® and FloSeal®) applied to healing nasal mucosa in the rabbit were found to be incorporated into the tissue leading to a lymphocytic reaction. This resulted in inflammation, scarring and fibrosis compared to the control sides which was left untreated (Maccabee *et al.,* 2003). Of note, FloSeal in one study was shown to increase the rate of granulation tissue and synechiae formation in the nose and sinuses after ESS (Chandra *et al.,* 2003). In contrast Merogel® has not been shown to have significants effect either beneficial or detrimental after ESS (McIntosh *et al.,* 2002; Rajapaksa *et al.,* 2005a).

In the present study no relationship was found between adhesion or synechiae formation and the use of AMICAR or normal saline. The incidence of adhesions and synechiae were similar in both normal saline and AMICAR treated sides.

4. Re-epithelialisation of the nasal cavity mucosa following full thickness mucosal injury with the subsequent use of AMICAR or AMICAR and methyl cellulose

The rate of re-epithelialisation as assessed by light microscopy of biopsy cross sections was almost complete at day 56. The use of AMICAR with or without methyl cellulose did not have any effect on the rate of re-epithelialisation compared to normal saline. Observation of mucosal biopsy cross sections under the light microscope did not show inflammation, foreign body reaction, tissue necrosis or fibrosis. These sections showed normal mucosal healing patterns in both normal saline and AMICAR treated sides. Therefore AMICAR is a haemostatic agent that does not interfere with normal wound healing and re-epithelialisation.

Previous studies on chronic sinusitis sheep have shown that the rate of re-epithelialisation in these animals was significantly slower compared to healthy sheep, the greatest difference seen at day 112 (Rajapaksa *et al.*, 2005b). In the present study all sheep showed near complete re-epithelialisation by day 112, with either treatment.

Fibrin sealants have been shown to have minimal interference with wound healing when applied to a wide variety of tissues such as bone, skin, gastrointestinal mucosa

and blood vessels (Jackson, 2001). While AMICAR is aqueous and can be sprayed over a surface, fibrin sealants are warmed and premixed during application via a double barrelled syringe.

5. Re-ciliation of the nasal cavity mucosa following full thickness mucosal injury and the subsequent use of AMICAR or AMICAR with methyl cellulose

The percentage area of the mucosal surface with mature cilia obtained by scanning electron microscopy showed that only about 6% of the area was ciliated with mature cilia at day 28 in both normal saline and AMICAR treated sides. The highest rate of re-ciliation with mature cilia was seen at day 84 on the normal saline treated side (71% vs 42%; p=0.04). Day 112 rate of re-ciliation appear to be lower in the saline treated side being 51% for saline treated side and 56% for AMICAR treated side. This difference is small and the rate of re-ciliation appears to be similar for saline treated side and AMICAR treated side. When AMICAR was used in combination with methyl cellulose, no significant difference was observed in the rate of re-ciliation compared to that of AMICAR alone or normal saline. As this finding was confined to one isolated time point it is unlikely to be of clinical significance.

In the previously studies sheep model of chronic sinusitis a statistically significant difference in the area of reciliation was observed at day 112 between the chronic sinusitis sheep and healthy sheep (Rajapaksa *et al.*, 2005b). This may reflect impaired re-ciliation in chronically infected sheep. As all sheep in the present study were infested with *O ovis* the rate of re-ciliation may have been slower than healthy animals.

Up to 45% of cilia seen in both sides at all time intervals were morphologically immature. There was no statistical difference between the area of immature cilia in normal saline treated side and AMICAR treated side for any time interval. This suggests that the reciliation process may be a dynamic process with repair and new epithelial cells regenerating throughout the period of study in both saline treated side and the AMICAR treated side.

The area of epithelium re-ciliated with both mature and immature cilia was found to be 98% by day 112 in the AMICAR treated side and 94% in saline treated side. However on day 84 the AMICAR treated side total ciliation reached 93% while the saline treated side was 67%. The total ciliation for all time points in the normal saline treated side was less than the AMICAR treated side. This suggests a complete recovery of the epithelium by day 112. However the saline treated side, up until day 112 may have had a slower rate of ciliary growth.

Re-ciliation of healing mucosa has not been studied following the use of topical haemostatic agents such as fibrin sealants. In addition, re-ciliation data is not available for antifibrinolytic amino acids such as amino caproic acid and tranexamic acid, although these have been used in oral and gastrointestinal bleeding (Mannucci 1998). Therefore the findings of the current study that show AMICAR does not significantly inhibit the rate of re-ciliation of regenerating nasal mucosa compared to normal saline is a novel finding. Of note, the scanning electron microscopic appearance of cilia was identical in both normal saline treated side and the AMICAR treated side. The use of a mucoadhesive did not affect the rate of re-ciliation or the morphology of cilia.

6. Epithelial height

In a healthy epithelium, epithelial height may be a reflection of cell division and proliferation. However, in inflamed or infected epithelia, the cells may swell and appear higher due to intracellular accumulation of water and other substances. In the present study, the biopsy specimens were taken from newly formed epithelia, and they may reflect cell division and repair as well as inflammatory signs as *Oestrus ovis* larvae were seen to continue to infest the sheep nasal cavities.

The initial preoperative epithelial height was similar to that obtained at days 28 and 56 post operatively in both normal saline and AMICAR treated animals. Under light microscopic observations by day 28 minimal inflammatory cells were seen. The epithelium consisted of morphologically normal pseudostratified columnar epithelial cells. There was a general trend for the epithelium to gain in height after day 56 in both normal saline and AMICAR treated sides, but a significant difference was only

observed between day 84 and day 112 biopsies, in both normal saline and AMICAR treated sides. Furthermore, AMICAR did not significantly affect the height of the epithelium compared to normal saline at any time interval.

In previously established sheep model of chronic sinusitis, the day 28 the epithelial height was shown to be significantly higher than in healthy sheep. It was also noted that day 56, 84 and 112 biopsies of chronic sinusitis sheep showed no significant increase in the epithelial height compared to that of healthy sheep (Rajapakse *et al.,* 2005a). They also reported a trend towards a decrease in epithelial height by day 112 in the sinusitis sheep. The present findings show a trend towards increasing epithelial height in all sheep from day 56. As the height measurement was taken from the basement membrane, only the true epithelial cell height was measured, thus excluding any inflammatory change such as oedema of the submucosal tissues. As shown in the previous study, a higher epithelial height may be expected in *O Ovis* infested sheep. As all sheep were infested on both nasal cavities, epithelial height was expected to be high. In addition, the full thickness mucosal injury created at the time of surgery may have rendered the newly forming epithelium to be more vulnerable to the pre-existing larval infestation and its effects. This may explain the gain of height after day 56, following a lag time presumably in keeping with a larval cycle and / or cell cycle. This may also reflect the time to achieve optimal levels of surface antibodies and other mucosal defences against the parasitic infestation.

7. Conclusions

The current study used a well established sheep model of chronic sinusitis, namely sheep chronically infested with *O ovis* larvae. The presence of tissue inflammation in the nasal mucosa was further quantified by light microscopic evidence of eosinophilia within the nasal mucosa as well as in the secreted mucous.

This study confirmed the presence of tissue and secreted eosinophilia in sheep infected with *Oestrus ovis* larvae. In order to confirm the eosinophilic inflammatory reaction resulting from the infestation, a minimum baseline level of 2 eosinophils per high power field was established. This finding will allow future studies based on the sheep model of chronic eosinophilic rhinosinusitis to confirm the presence of *Oestrus*

ovis induced sinusitis. This is a minimally invasive diagnostic test, performed following sedation and local anaesthetic spray. Eosinophilic mucus samples can be obtained using a cell brush and a small biopsy of the lateral nasal wall can be obtained using biopsy scissors. This diagnostic test can be utilised to confirm infestation with *Oestrus ovis* larvae particularly when the larvae are found within the sinuses and not visualised with the endoscope. In addition this can be used to exclude subjects with minimal or no eosinophilia in studies requiring sheep with eosinophilic sinusitis.

The topical haemostatic agent used in the study, AMICAR, a lysine analogue, showed a significant reduction in the grade of surgical field bleeding when used on the lateral nasal wall inferior to the middle turbinate attachment. When data for all sites were pooled there was a non significant trend towards a shorter time to achieve haemostasis with AMICAR. The use of methyl cellulose as a mucoadhesive did not achieve the desired effect of improved haemostasis by lengthening contact time between AMICAR and the wound. Furthermore there was no correlation between the degree of bleeding and the degree of inflammation as reflected by eosinophilia in the mucosa.

In the post operative period, no correlation was found between application of AMICAR or saline and synechiae or adhesion formation in previously injured sites.

Re-epithelialisation was near complete by day 56, regardless of the treatment received. In contrast re-ciliation was never seen to be complete, and by day 112 only about half the observed area showed mature cilia. No significant effects were observed due to AMICAR on re-epithelialisation or on re-ciliation.

This study was a pilot study to establish whether AMICAR would be worth further investigation as a possible topical haemostatic agent. If this study was carried out with greater numbers the other variables such as the larval burden could have been better controlled and the statistical analysis may have revealed different results.

The advantage of using AMICAR as a spray was that a uniform coverage could be obtained using the delivery system utilised in this study. The use of a mucoadhesive significantly increased the viscosity, and this was thought to compromise the delivery.

The other advantage of using AMICAR is that it is biodegradable and does not incite an immune or foreign body reaction. As it is a synthetic analogue there is no risk of disease transmission.

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