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## Article

# Equine Corneal Wound Healing using Mesenchymal Stem Cell Secretome: Case Report

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**Simple Summary:** This case report describes the successful treatment of a non-healing corneal ulcer in a 28-year-old mare using the secretome derived from adipose tissue-derived mesenchymal stem cells (ASCs). Despite initial conventional treatment with antibiotics, anti-inflammatory drugs, and surgical debridement, the corneal ulcer failed to heal properly. As an alternative regenerative approach, the ASC secretome, containing trophic factors, cytokines, and extracellular vesicles, was topically administered to the affected eye. Remarkably, within one week of secretome treatment, the clinical signs resolved, the corneal ulcer exhibited complete re-epithelialization, regained transparency, and reduced neovascularization. No recurrence was observed during the 1.5-year follow-up period. This highlights the potential of ASC secretome as a novel cell-free therapy for treating refractory corneal ulcers in horses.

**Abstract:** Corneal ulcers are a common and potentially vision-threatening condition in horses that can be challenging to treat with conventional therapies alone. This case report describes the successful treatment of a non-healing corneal ulcer in a 28-year-old Hispano-Bretón mare using the secretome derived from adipose tissue-derived mesenchymal stem cells (ASCs). Despite initial treatment with antibiotics, anti-inflammatory drugs, and surgical debridement, the corneal ulcer failed to heal properly, exhibiting persistent epithelial defects and stromal complications. As an alternative regenerative approach, the ASC secretome, a rich source of trophic factors, cytokines, and extracellular vesicles, was topically administered to the affected eye. Remarkably, within one week of secretome treatment, the clinical signs of blepharospasm and epiphora resolved, and the corneal ulcer exhibited complete re-epithelialization, regained transparency, and reduced neovascularization. No recurrence was observed during the 1.5-year follow-up period. This case highlights the potential therapeutic benefits of ASCs secretome in promoting corneal wound healing and suggests its promise as a novel cell-free therapy for treating refractory corneal ulcers in horses.

**Keywords:** corneal non-healing ulcer; horses; adipose tissue mesenchymal stem cells; secretome

## 1. Introduction

Corneal ulcer is a common and potentially serious condition in horses. Damage to the cornea, resulting in loss of epithelial cells with exposed corneal stroma, creates an ulcer on the corneal surface [1]. Squinting (blepharospasm) or keeping an eye shut and tearing (epiphora) are often the first signs owners will notice in pets with corneal ulcers. Fluorescein stain uptake in the ulcerated region confirms the diagnosis of an ulcer. Categorization of the ulcer is based on a combination of clinical signs and visual inspection of the eye [1,2].

Indolent corneal ulcers, also known as refractory ulcers, "boxer" ulcers, spontaneous chronic corneal epithelial defects (SCCEDs), and recurrent erosions, are a special type of superficial ulcer that fails to resolve through the normal wound-healing process [3]. Hallmark clinical and histologic features of SCCEDs include a superficial corneal ulcer that (1) does not extend into the stroma; (2) is associated with redundant, non-adherent corneal epithelial borders that may be associated with an acellular hyaline zone in the anterior stroma; (3) persists for weeks or months if not adequately addressed; and (4) may or may not include neovascularization and edema [4–6].

The underlying pathophysiology of SCCEDs is not completely understood, and there is some thought that these ulcers may be the shared end result of a variety of pathways. Ultimately, indolent corneal ulcers result from dysmaturation of corneal epithelia that do not properly attach to the underlying stroma of the eye, creating a lesion composed of loose, poorly adhered epithelia overlying corneal stroma (lipping) [5,7].

Traditional treatments for corneal ulcer in horses include antibiotics, anti-inflammatory medications, and surgery [1,7]. However, these treatments can be costly, time-consuming, and sometimes ineffective [8]. In recent years, regenerative medicine has emerged as a promising approach for treating various conditions, including corneal wounds [9]. One of the most promising alternatives developments is the use of mesenchymal stem cells (MSCs) [10–12]. In MSCs therapy, stem cells are harvested from the patient or from a donor and then cultured in a laboratory. The MSCs are then administered to the patient to promote healing and tissue regeneration. However, recent studies have shown that the beneficial effects of MSCs therapy may not be solely due to the stem cells themselves, but rather to the secretion of various factors, known as the secretome also called conditioned medium (CM) by the MSCs [13,14]. MSCs secretome refers to the collection of bioactive molecules, including growth factors, cytokines, and extracellular vesicles (EVs), secreted by these cells. The secretome of MSCs has gained significant attention due to its potential to modulate the wound healing process by regulating various cellular processes, such as inflammation, angiogenesis, and extracellular matrix remodeling [15]. Furthermore, the use of the secretome eliminates the need for direct cell transplantation, reducing the risks associated with cell-based therapies, such as immune rejection and potential tumorigenicity [16].

This case report describes a novel approach to treating a corneal wound in a Hispano-bretón mare using the secretome derived from adipose tissue mesenchymal stem cells (ASCs). We describe the clinical presentation, treatment protocol, and outcome, highlighting the potential of ASC secretome as a promising therapeutic strategy for corneal wound healing in veterinary ophthalmology.

## 2. Case Description

A 28-year-old mare of the Hispano Breton breed kept in a paddock with two other horses. The general examination of the animal does not show any alteration, the ocular examination shows blepharospasm and moderate blepharitis, moderate/mild epiphora and the tear secretion is serous. The pupil appears miotic, with moderate corneal edema and localized corneal neovascularization in the dorsal region, with conjunctival hyperemia. Fluorescein staining revealed the presence of a corneal ulcer of about 7 mm in diameter, apparently uncomplicated.

Treatment was started with tobramycin in eye drops (Tobrex®) every 4 hours, atropine (Colircusi® Atropina 1%) in eye drops every 12 hours during the first 48 hours and then as needed, autologous serum every 2 hours in the morning and every 3 hours in the afternoon and intravenous flunixin meglumine (Finadyne®) at a dose of 1.1mg/kg every 24 hours for two days.

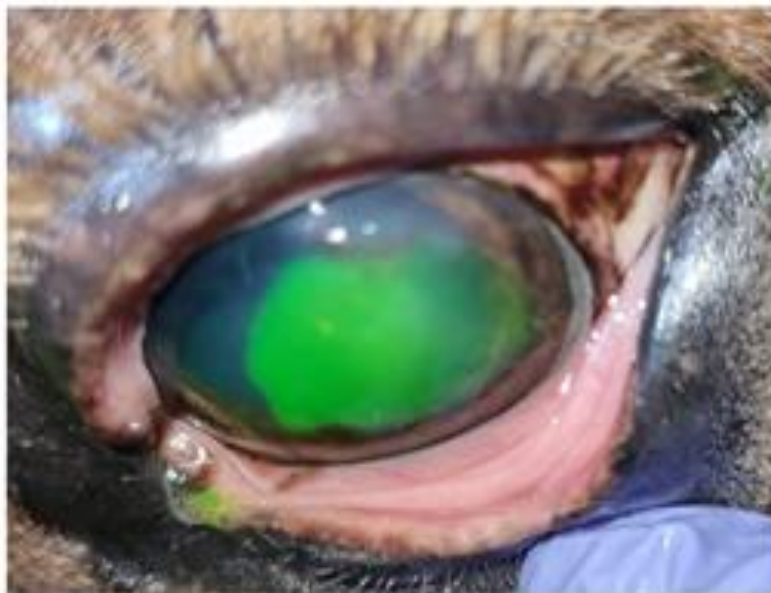
A first review after 4 days showed improvement of blepharospasm and corneal edema, although epiphora persisted. Fluorescein staining revealed dye infiltration under the epithelial borders of the lesion. Slit-lamp biomicroscopic examination and fluorescein staining revealed detached epithelial edges (Figure 1), confirming the diagnosis of indolent/nonhealing corneal ulcer. The examination was completed, ruling out the existence of a foreign body, and debridement was performed. After sedation with detomidine (Domosedan®) and butorphanol (Torbugesic Vet®), in addition to topical anesthesia, mechanical debridement of the lesion was performed with a Algerbrush 3.5 mm®

diamond bur. The non-adherent epithelium was removed along the entire contour of the lesion and the lesion bed was also reamed.



**Figure 1.** Fluorescein test stain showed corneal injury with fluorescein infiltration under the edges of the epithelium.

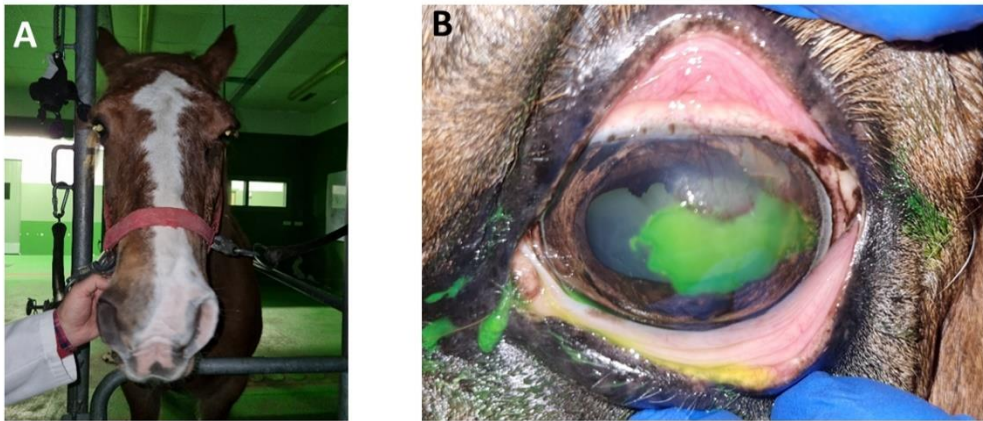
The result was a significant increase in the extent of the corneal ulcer. It occupied 2/3 of the corneal surface (Figure 2). The previously prescribed medical treatment with systemic flunixin meglumine was maintained for two days.



**Figure 2.** Aspect of the lesion after the first debridement, a significant increase in the extent of the corneal ulcer is observed.

At the next examination 1 week later, moderate blepharospasm (Figure 3.A) was observed. The lesion was fluorescein positive (Figure 3.B) and was accompanied by significant cellular and vascular infiltration in the superotemporal quadrant of the cornea. He underwent another debridement with diamond burr. The result was a reduction of the ulcerated surface, with respect to the previous debridement. Medical treatment was maintained, in this case with flunixin meglumine for 3 days.





**Figure 3.** Aspect of the lesion at the second examination 1 week later. 3.A) Moderate blepharospasm in the left eye was observed. 3.B) After the second debridement the lesion was fluorescein positive.

One week later, at the second examination, the clinical signs of blepharospasm and epiphora were still present, with significant cellular and vascular infiltration of the cornea. Fluorescein staining was negative but the epithelium did not appear to be completely adherent to the stroma on biomicroscopic examination, a touch with the Kimura spatula revealed sliding of the epithelium over the corneal stroma. Debrided, the final result is a lesion of greater extent than after the previous debridement (Figure 4). There is no improvement from the previous consultation.



**Figure 4.** After all antibiotic and anti-inflammatory treatment and third debridement, the lesion persists without significant improvement respect to the previous debridement.

After this conventional treatment without significant evolutions we consider the need to modify the therapeutic strategy in order to promote proper re-epithelialization, the patient underwent therapeutic intervention with ASCs secretome. Adipose tissue was collected from the subcutaneous fat deposits of healthy horses during routine surgical procedures. The ASCs were isolated from the adipose tissue through enzymatic digestion with collagenase at 37°C for 2 hours. The purified ASCs were cultured until confluence. When 80% confluence was reached, ASCs were maintained in serum-free DMEM medium for 48 hours to produce CM. The CM was collected and centrifuged to remove cellular debris. The cell-free CM, containing the secretome of proteins, cytokines, and other factors secreted by the equine ASCs, was concentrated using ultrafiltration membranes.

After informing the owners of the experimental treatment condition and obtaining their consent we administered in drops topically the secretome obtained from ASCs every 4 hours.

One week later the clinical signs of blepharospasm and epiphora had disappeared, the lesion was fluorescein negative (Figure 5), on biomicroscopic examination the lesion appeared well re-epithelialized, yet swabbing was performed on the surface of the lesion, which revealed adherence of the epithelium to the corneal stroma.



**Figure 5.** Negative fluorescein test stain showed absence corneal injury after secretome therapy. The cornea has regained transparency, reduced neovascularization and disappeared cellular infiltration.

The re-epithelialized area had regained transparency, cellular infiltration had disappeared, and improved corneal neovascularization was observed. The administration of secretoma was maintained for 3 more days and the patient was discharged with no recurrence in a year and a half after treatment.

#### 4. Discussion

The findings from this case report highlight the potential therapeutic benefits of using mesenchymal stem cell (MSC) secretome, specifically from adipose-derived MSCs (ASCs) [17], for treating non-healing corneal ulcers in horses [10,18]. Despite initial conventional treatment with antibiotics, anti-inflammatory drugs, and debridement, the corneal ulcer in this Hispano-Bretón mare failed to heal properly, exhibiting signs of persistent epithelial defects.

The lack of significant improvement with standard therapy prompted the exploration of an alternative regenerative approach using the secretome derived from cultured equine ASCs [8]. The ASCs secretome, a rich milieu of trophic factors, cytokines, and extracellular vesicles, has been shown to modulate various cellular processes crucial for wound healing, including inflammation, angiogenesis, and extracellular matrix remodeling [19,20].

By harnessing the paracrine effects of the ASC secretome, topical administration facilitated the re-epithelialization of the corneal ulcer and resolution of associated clinical signs within a week of treatment initiation.

These findings corroborate previous studies that have demonstrated the therapeutic potential of MSC secretomes in promoting corneal wound healing [21]. For instance, a study by Saccu et al. reported significantly faster healing times and reduced complications in corneal ulcers treated with bone marrow-derived MSC secretome compared to a placebo group [22]. Similarly, Cunha et al. [23] observed enhanced corneal re-epithelialization and reduced inflammation in rabbits treated with ASC secretome after corneal alkali burns. However, there is a paucity of research investigating the

use of stem cell therapies for the treatment of corneal ulcers in veterinary medicine. This gap in knowledge is even more pronounced when considering the equine species. To date, no studies have evaluated the therapeutic effects of stem cell secretomes, specifically those derived from ASCs, on corneal ulcers in horses.

The regenerative capacity of MSC secretomes is attributed to their ability to modulate the wound microenvironment through various mechanisms [20]. The secreted factors can exert anti-inflammatory effects by regulating the activity of immune cells and reducing oxidative stress. Additionally, the secretome contains growth factors and cytokines that stimulate the proliferation and migration of corneal epithelial cells, facilitating re-epithelialization [24,25]. Furthermore, the extracellular vesicles present in the secretome can transfer bioactive molecules, such as proteins, lipids, and nucleic acids, to target cells, influencing their behavior and promoting tissue repair [26].

While the results from this case report are promising, it is important to acknowledge the limitations of a single case study. Further controlled studies with larger sample sizes are necessary to validate the efficacy and safety of ASCs secretome therapy for treating corneal ulcers in horses. Additionally, optimizing the dosing regimen, administration route, and formulation of the secretome could potentially enhance its therapeutic effects.

## 5. Conclusions

Overall, these findings suggest that ASCs secretome therapy may be a safe and effective novel regenerative therapy for treating non-healing corneal ulcers in horses. The topical application of the ASCs secretome facilitated corneal re-epithelialization and resolution of clinical signs in a case refractory to conventional treatment. These findings contribute to the growing body of evidence supporting the use of MSC secretomes as a cell-free, minimally invasive approach for promoting tissue repair and regeneration in veterinary ophthalmology.

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**Data Availability Statement:** Data will be made available on request.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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