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TEARS. THERE IS SO MUCH MORE TO THEM THAN YOU THINK

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The role of tears in the ocular surface microenvironment (OSM)

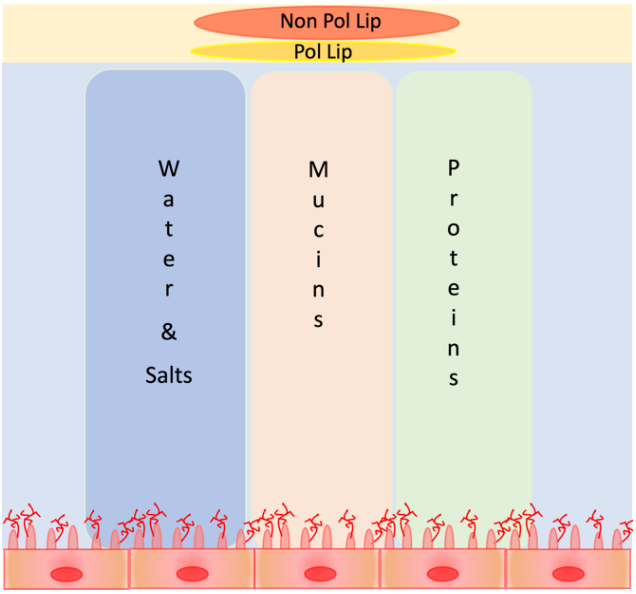
The ocular surface (OS) is a complex system with several components continuously interacting with each other to maintain a balanced OSM. Tears play a central role to keep it steady, their quality and quantity being conditioning factors for the OS health. While examining the OS to make a diagnosis, the veterinary ophthalmologist must always consider all the components of the OSM as part of a whole. It is important to know if meibomian glands (MGs) are normal and able to produce a tear film (TF) lipid layer (LL) of appropriate thickness and composition, if the secretion of lacrimal glands (LGs) is within the normal parameters, if the corneal and conjunctival epithelial surfaces have the expected wettability. It is important to evaluate if the eyelids' shape and function are adequate to distribute the TF with blinking, if ambient agents and local microbiome interfere with OSM homeostasis.

The Lacrimal Functional Unit[1]

The lacrimal functional unit (LFU) is composed of the lacrimal glands, the OS and the interconnecting innervation.
The nerves of the OS convey thermal and chemical stimuli, mechanical and pain sensing through the afferent ophthalmic branch of the trigeminal nerve to the pons. The efferent autonomic fibers stimulate lacrimal glands, meibomian glands and goblet cells to produce a perfect balance of the tear film components. The facial nerve controls blinking to spread tears over the OS.

The Tear Film

The TF is a hydrated **mucin gel** whose mucin concentration decreases with distance from the epithelial surface. It interacts with the corneal and conjunctival epithelium via the membrane-spanning mucins.



The **superficial LL** is composed of polar and non-polar lipids mainly produced by the MGs with holocrine secretion.
Polar lipids are oriented perpendicularly, with their hydrophobic tails immersed in the upper nonpolar lipid sublayer, and their polar heads exposed to the aqueous layer to create an interface that maintains TF stability.[2]
Nonpolar lipids form the upper portion of the lipid layer in contact with air. If they are spread over the aqueous layer without a polar lipid interface, they are unstable, collapse and form lipid droplets with consequent evaporation of the aqueous portion of the TF.
The LL limits the evaporation of the aqueous phase and stabilizes the TF by lowering surface tension. Hence, meibomian lipids are essential for the maintenance of OS health and integrity.
To assess the MGs anatomical and functional characteristics is of primary interest for the differential diagnosis of diseases affecting the OS both of humans and animals.
Meibomian gland dysfunction (MGD) is the main cause of lipid layer disorders in companion animals with consequent increased TF evaporation.
The **aqueous component** of tears is secreted by the orbital and third eyelid lacrimal glands.

The aqueous phase of the tear film is a mucin gel, loaded with water, secreted soluble mucins, electrolytes, glucose, leukocytes, metabolites (aminoacids, urea, glucose, lactate) and proteins like cytokines, immunoglobulins, enzymes, and growth factors.
Mucins secreted by the conjunctival goblet cells (**secretory mucins**) are large, high molecular weight glycoproteins with protective functions for the ocular surface, like lubrication, barrier formation and hydration.
TF **proteins** derive from the lacrimal glands and, via leakage, from conjunctival blood vessels.

The Ocular surface epithelia
The OS is protected by a continuous sheet of epithelium extending from the cornea to the lid margins, involving the conjunctiva over the anterior globe, the third eyelid and the eyelids.
When the eye is open, TF evaporation becomes a potential stressing factor that may desiccate the OS and alter the epithelium with loss of wettability.
OS mucins in the glycocalix of the most superficial cell layer (**transmembrane mucins**) are synthesized by corneal and conjunctival epithelia and contribute to the epithelial barrier, to maintain OS wettability and tear film stability by anchoring secreted mucins.

Ocular surface homeostasis
All components of the tear film lacrimal functional unit play an important role in maintaining the ocular surface homeostasis. Loss of ocular surface homeostasis may be the result of diseases affecting one or more components of the OSM. In companion animals, eyelids, conjunctiva, meibomian glands, lacrimal glands and ocular surface epithelia are more often affected.
The main consequences are tear film instability with increased evaporation (evaporative dry eye: EDE) and tear film deficiency (aqueous deficient dry eye: ADDE).
EDE is the direct outcome of several pathological conditions affecting dogs and cats. The most frequent are:

- MGD, with altered meibum secretion and composition
- conjunctival diseases involving goblet cells and, as a consequence, altering the secretory mucin component
- corneal epithelial defects with cellular metaplasia and loss of OS wettability
- eyelid functional and anatomical defects with insufficient or altered blinking

ADDE is the outcome of decreased tear production secondary to any disease affecting the lacrimal glands.
In both EDE and ADDE cases TF deficiency increases the concentration of electrolytes in the muco-aqueous layer and, as a consequence, TF osmolarity raises triggering a vicious inflammatory cycle. Firstly, an innate reaction with production of inflammatory mediators (MMPs, cytokines) is established, and then the immune mediated reaction with the consequent cascade of events due to T and B cells activation occurs.

Other components of the OSM
Other than the LFU components, ambient agents, microbiome, immune cells and hormones continuously interact with the LFU and are important conditioning factors to be considered by clinicians to better understand the TF disorders.[3]
The OS is a potential battlefield with a delicate and complex equilibrium. A loss of balance gives rise to compensatory processes but may also trigger a disease process with serious consequences, activation of local innate and immune reactions, rise of TF osmolarity and loss of control of the inflammatory process.

Main factors influencing the TF homeostasis
- The OS epithelia. They are exposed to the external environment but have tight intercellular junctions that resist the entry of noxious pathogens. OS wettability, necessary to maintain TF stability, depends upon the epithelial integrity.
- The OS nerve fibers derived from the branches of the trigeminal nerve. They maintain the action of blinking and tear reflex and contribute to the major components of the tear film. Nerve endings

secrete neurotransmitters and nerve growth factors to maintain the epithelial integrity, proliferation and wound healing.[4]
- The eyelids to distribute TF over the OS. Early identification and management of eyelid abnormalities is mandatory to improve TF quality and prevent EDE and ADDE.
- The eye-associated lymphoid tissue (EALT) for immune protection of the ocular surface and its mucosal adnexa. It is anatomically continuous from the lacrimal gland throughout the conjunctiva and the lacrimal drainage-associated lymphoid tissue (CALT and LDALT, respectively). It consists of a diffuse lymphoid tissue of T lymphocytes and IgA-secreting plasma cells and of lymphoid follicles.[5]
- Ambient temperature and humidity. High temperature and low ambient relative humidity may increase TF evaporation, mainly if blinking frequency is not increased to maintain a protective TF.
- Indoor and outdoor air pollution may contaminate the TF and increase evaporation.
- Microbiome. The loss of TF-OS integrity in EDE and ADDE promotes the flora to exert its pathogenic effect and activates triggering of the innate immune response.[3]

In case of loss of TF-OS homeostasis due to imbalance of OSM components, it is necessary to perform diagnostic tests to reach a correct diagnosis and choose the most appropriate treatment.

Suggested readings

1. Stern, M.E. et al. The pathology of dry eye: The interaction between the ocular surface and lacrimal glands. Cornea 1998, 17, 584–589
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3. Xiaobo Zhang et al. Dry Eye Management: Targeting the Ocular Surface Microenvironment. Int. J. Mol. Sci. 2017, 18, 1398-1426
4. Belmonte C et al. TFOS DEWS II pain and sensation report. The Ocular Surface 15 (2017) 404-437
5. Knop E, Knop N. Anatomy and immunology of the ocular surface. In: Niederkorn JY, Kaplan HJ (eds): Immune Response and the Eye. Chem Immunol Allergy. Basel, Karger, 2007, vol 92, pp 36–49

ABBREVIATIONS
ADDE: aqueous deficient dry eye
CALT: conjunctiva associated lymphoid tissue
EALT: eye associated lymphoid tissue
EDE: evaporative dry eye
LDALT: lacrimal drainage associated lymphoid tissue
LFU: lacrimal functional unit
LL: lipid layer
MGs: meibomian glands
MGD: meibomian gland dysfunction
OS: ocular surface
OSM: ocular surface microenvironment
STT: Schirmer tear test
TF: tear film