



## TFOS DEWS II Sex, Gender, and Hormones Report



David A. Sullivan, PhD <sup>a,1,\*</sup>, Eduardo M. Rocha, MD, PhD <sup>b</sup>, Pasquale Aragona, MD, PhD <sup>c</sup>,  
 Janine A. Clayton, MD <sup>d</sup>, Juan Ding, OD, PhD <sup>e</sup>, Blanka Golebiowski, PhD <sup>f</sup>,  
 Ulrike Hampel, MD <sup>g</sup>, Alison M. McDermott, PhD <sup>h</sup>, Debra A. Schaumberg, ScD, OD <sup>i,j</sup>,  
 Sruthi Srinivasan, PhD <sup>k</sup>, Piera Versura, BSD <sup>l</sup>, Mark D.P. Willcox, PhD, DSc <sup>f</sup>

<sup>a</sup> Schepens Eye Research Institute, Massachusetts Eye and Ear, Harvard Medical School, Boston, MA, USA

<sup>b</sup> Ribeirão Preto Medical School, University of São Paulo, São Paulo, Brazil

<sup>c</sup> Department of Biomedical Sciences, Ocular Surface Diseases Unit, University of Messina, Messina, Sicily, Italy

<sup>d</sup> National Institutes of Health Office of Research on Women's Health, Bethesda, MD, USA

<sup>e</sup> Schepens Eye Research Institute, Massachusetts Eye & Ear, Department of Ophthalmology, Harvard Medical School, Boston, MA, USA

<sup>f</sup> School of Optometry and Vision Science, University of New South Wales, Sydney, Australia

<sup>g</sup> Department of Ophthalmology, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany

<sup>h</sup> The Ocular Surface Institute, College of Optometry, University of Houston, Houston, TX, USA

<sup>i</sup> Harvard School of Public Health, Boston, MA, USA

<sup>j</sup> University of Utah School of Medicine, Salt Lake City, UT, USA

<sup>k</sup> Centre for Contact Lens Research, School of Optometry, University of Waterloo, Ontario, Canada

<sup>l</sup> Department of Specialized, Experimental, and Diagnostic Medicine, University of Bologna, Bologna, Italy

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## ABSTRACT

One of the most compelling features of dry eye disease (DED) is that it occurs more frequently in women than men. In fact, the female sex is a significant risk factor for the development of DED. This sex-related difference in DED prevalence is attributed in large part to the effects of sex steroids (e.g. androgens, estrogens), hypothalamic-pituitary hormones, glucocorticoids, insulin, insulin-like growth factor 1 and thyroid hormones, as well as to the sex chromosome complement, sex-specific autosomal factors and epigenetics (e.g. microRNAs).

In addition to sex, gender also appears to be a risk factor for DED. "Gender" and "sex" are words that are often used interchangeably, but they have distinct meanings. "Gender" refers to a person's self-representation as a man or woman, whereas "sex" distinguishes males and females based on their biological characteristics. Both gender and sex affect DED risk, presentation of the disease, immune responses, pain, care-seeking behaviors, service utilization, and myriad other facets of eye health.

Overall, sex, gender and hormones play a major role in the regulation of ocular surface and adnexal tissues, and in the difference in DED prevalence between women and men. The purpose of this Subcommittee report is to review and critique the nature of this role, as well as to recommend areas for future research to advance our understanding of the interrelationships between sex, gender, hormones and DED.

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## 1. Introduction

One of the most compelling features of dry eye disease (DED) is that it occurs more frequently in women than men [1,2]. In fact, the

female sex is a significant risk factor for the development of DED [1,2]. That such a sex-related variation exists in the prevalence of an eye disease, or any other ocular function, should not be a surprise. Sex-related differences are present in almost every cell, tissue and organ system of the body, including those associated with circulation, respiration, digestion, renal function, metabolism, and neural and endocrine activity [3]. Indeed, since 1875, more than 650,000 scientific reports have been published which address the basic and/or clinical impact of sex.

\* Corresponding author.

E-mail address: [david.sullivan@schepens.harvard.edu](mailto:david.sullivan@schepens.harvard.edu) (D.A. Sullivan).

<sup>1</sup> Subcommittee Chair

The influence of sex on the eye has been known for almost 2500 years. Conditions associated with sex differences include blepharospasm, eyelid edema, conjunctivitis, keratitis, herpetic reactivation, corneal ulcer, iritis, cataract, glaucoma, amblyopia, scotoma, optic neuritis, optic nerve atrophy and blindness [4]. As stated in a 1888 monograph addressing clinical ophthalmology “males are by no means as prone to diseases of the eye from sexual causes as females.” [5].

Since that time, investigators have identified numerous sex-related differences in the eye, and many of these variations have been attributed to the effects of sex steroids (i.e. androgens, estrogens and progestins). For example, sex-associated differences have been identified in, and sex steroids have been shown to act on, the meibomian gland, lacrimal gland, conjunctiva, cornea, anterior chamber, iris, ciliary body, lens, vitreous and retina. These hormone actions appear to be mediated through classical, and possibly membrane, receptors and impact multiple structural and functional aspects of the eye. These include tissue morphology, gene expression, protein synthesis, epithelial cell dynamics, aqueous tear output, lipid production, mucous secretion, tear film stability, blink rate and immune function [6–17]. Sex and/or sex steroids have also been linked to the development, progression and/or treatment of many ocular conditions, including DED, meibomian gland dysfunction (MGD), wound healing, keratoconjunctivitis, corneal transplant rejection and corneal pathologies [18–23].

These sex-related differences in the eye are not due solely to the effects of sex steroids. As detailed in this report, hypothalamic-pituitary hormones, glucocorticoids, insulin, insulin-like growth factor 1 and thyroid hormones also contribute notably to these sex-associated variations. In addition, sex-related differences may arise from the sex chromosome complement, including differences in parent-of-origin effects, X chromosome gene dosage (e.g. X-inactivation) and genes in the non-recombining region of the Y chromosome [24–30], as well as from sex-specific autosomal factors and epigenetics (e.g. microRNAs [miRNAs], DNA methylation and acetylation, histone modifications) [24,31,32].

It is important to note that we use the word sex for a reason. Although sex and gender are often used interchangeably throughout the literature, they have distinct meanings. As stated by the Institute of Medicine [3], sex refers to the classification of living things, generally as male or female, according to their reproductive organs and functions assigned by chromosomal complement. Gender refers to a person's self-representation as male or female, or how social institutions respond to that person based on the individual's gender presentation. Gender is rooted in biology and shaped by environment and experience. In other words, sex distinguishes males and females based on their biological characteristics. Gender, in turn, reflects socially constructed characteristics such as behaviors and expectations related to being a man, masculine, or being a woman, feminine. Furthermore, gender is dynamic, context-related and operates on a spectrum.

The correct and consistent usage of the terms sex and gender across scientific disciplines promotes the accurate assessment, measurement, and reporting of differences between men and women. In most studies of nonhuman animals the term sex should be used. The purposeful integration of considerations of sex and gender in health and disease throughout the scientific community will facilitate uptake by policy makers and dissemination to the general public.

In effect, both sex and gender affect health and disease, as well as patients' perceptions about their health. In addition, gender affects individuals' access to and interactions with the health care

system. Many health disparities<sup>2</sup> are associated with gender [33]. Eye-health disparities arise from a multitude of causes, some of which are known and some of which remain to be determined. Disparities arise from a range of influences that are biological, behavioral/perceptual, cultural, and societal. Therefore, in this report, we consider both gender and sex — terms that are distinguishable but intertwined as they both have pronounced effects on health and on health disparities. Gender and biological sex affect DED risk, presentation of the disease, immune responses, pain, care-seeking behaviors, service utilization, and myriad other facets of eye health [33].

Overall, sex, hormones and gender play a major role in the regulation of ocular surface and adnexal tissues, and in the difference in DED prevalence between women and men. The purpose of this Subcommittee report is to review and critique the nature of this role, as well as to recommend areas for future research to advance our understanding of the interrelationships between sex, gender, hormones and DED.

## 2. Sex and DED

### 2.1. Does sex matter?

Sex does matter. Sex-related differences are extremely important, as they directly or indirectly influence numerous physiological and pathological functions in the body. In the past significant attention has been focused on sex-based variations at the societal and whole organism levels. However, researchers' attention towards sex-associated differences at the basic *cellular* and *molecular* levels has been inadequate [3].

To address this lack of understanding, the Institute of Medicine commissioned a six-volume report to address our knowledge of biological sex differences and to identify barriers to the conduct of research in this area [3]. The conclusions and recommendations from this report, entitled “Exploring the Biological Contributions to Human Health: Does Sex Matter,” are very relevant for our understanding, now and in the future, of sex, hormones, gender and DED.

#### 2.1.1. Sex matters

The Institute of Medicine reported three conclusions:

- **Sex (male or female) matters.** Sex is an important basic human variable. Being male or female should be taken into consideration when designing and analyzing studies in all areas and at all levels of health-related and biomedical research. Individual genetic and physiological constitutions, combined with an individual's interaction with environmental factors and experiential factors, influence differences in health and illness. The occurrence, frequency and severity of diseases vary between males and females. These sex differences appear to be due to the effects of hormones, as well as other factors (e.g. genes).
- **The study of sex differences is evolving into a mature science.** There is now sufficient knowledge of the biological basis of sex-related differences to validate their scientific study and to permit the generation of experimental hypotheses.
- **Barriers to the advancement of knowledge about sex differences in health and illness exist and must be eliminated.**

<sup>2</sup> The term *health care disparities* refers to differences in access to or availability of facilities and services. *Health status disparities* refers to the variation in rates of disease occurrence and disabilities between socioeconomic and/or geographically defined population groups. (Both definitions are from the 2009 Medical Subject Headings (MeSH)).

**Table 1**  
Summary of barriers to progress in research on sex differences.

Item	Barrier
Terminology use in literature	•Inconsistent use of the terms sex and gender
Research tools and resources	•Studies with more complex experimental designs, model systems and additional resources are lacking. •Information on sex differences and sex of origin of cell and tissue culture material is lacking in the literature. •Lack of data from longitudinal studies about different diseases, disorders, and conditions across the life span. •Lack of consideration of endocrine status or hormonal variability.
Interdisciplinary and collaborative research	•Interpretation and application of federal regulations is not uniform. •Interdisciplinary research, training and translational research is lacking in the study of sex differences.
Non-health-related implications of research on sex differences in health	•Lack of awareness that the consequences of genetics and physiology may be open to change. •Discrimination based on identified sex differences.

Data are adapted from the Institute of Medicine report [3].

Scientists are confronted with a broad array of barriers when trying to conduct research on the role of sex differences in health and disease. These barriers, as summarized in Table 1 [3], encompass ethical, financial, sociological, and scientific considerations, and should be eliminated.

### 2.1.2. Every cell has a sex, sex begins in the womb, sex affects behavior and perception, and sex affects health

The Institute of Medicine report highlighted several findings:

- **Every cell has a sex.** Advances in molecular biology have identified the genetic and molecular basis of many sex-related differences in health and human disease, some of which appear due to the sexual genotype—XX in the female and XY in the male. Genes on these chromosomes can be expressed differently between males and females because of the presence of either one or two copies of the gene and because of different meiotic effects, X-chromosome inactivation, and genetic imprinting. The inheritance of either a male or female genotype is also influenced by the source (maternal or paternal) of the X chromosome. The different roles of the sex chromosome genes could explain X-chromosome-linked diseases, as well as the heterogeneous expression of some diseases within and between the sexes. There are multiple ubiquitous differences in the basic cellular biochemistries of males and females that can impact an individual's health, and these may be attributed to hormonal and genetic differences between the two sexes.
- **Sex begins in the womb.** Sex differences in human health and disease take place throughout the lifespan. Some originate in the intrauterine environment, others in the prenatal period, pre-puberty and puberty. Collectively, sex-related changes during these periods lay a framework for biological differences that persist through life and contribute to the variable onset and progression of disease in males and females. Consequently, it is important to research sex differences at all stages of the life cycle.
- **Sex affects behavior and perception:** Genetic and physiological differences, when combined with environmental factors, lead to behavioral and cognitive differences between males and females. Sex-related differences in brain organization, cognitive ability, pain perception, behavior and gender identity should be studied at all points in the lifespan. The sexual dimorphism in behavior, cognition, and perception appears to be due to hormones, genetics and other factors.
- **Sex affects health.** Males and females may have different patterns of illness and lifespans. Understanding the bases of these sex-related differences, as well as any similarities, is important to developing new approaches to the prevention, diagnosis, and treatment of diseases (e.g. DED).

### 2.1.3. Recommendations for better understanding of sex differences in health and disease

The Institute of Medicine report made a number of recommendations to advance our understanding of sex differences in health and disease. Several of these are as follows:

- **Identify** the roles of X- and Y-chromosome linked genes in somatic and germ-line cells, and determine with ethical research the impact of genetic sex differences on biological organization and disease susceptibility.
- **Include** sex as a variable in basic research, in order to reveal how sex-related differences influence health, disease and longevity.
- **Select** animal models for research that mirror human sex differences and are relevant for the human condition being addressed.
- **Evaluate** natural genetic variability, disorders of sex differentiation, reproductive status and environmental influences to gain a better understanding of human health.
- **Examine** sex-related differences and similarities for all human diseases that affect both sexes.
- **Determine** and disclose the sex of origin of cells and tissues used in biological research.

In summary, sex-related differences need to be systematically studied and elucidated, in order to advance our knowledge of their biological contributions to human health and disease. Such research is very important to permit understanding of why the female sex is a risk factor for the development of DED.

## 2.2. Epidemiology of sex differences in DED

### 2.2.1. Sex differences in prevalence and incidence of DED

Female sex is an established risk factor for DED-related autoimmune diseases such as Sjögren syndrome [34]. Female sex is also among the most widely studied and consistently identified risk factors for DED throughout the world. It is best studied in population-based epidemiological studies, since differences in care seeking behavior between women and men could influence associations in clinic-based studies (see Section 4). Among the larger epidemiological studies in North America, two parallel studies among over 39,000 women (Women's Health Study) [18] and 25,000 men (Physicians' Health Studies) [35] in the United States, showed a statistically significant age-adjusted 70% increase in risk of DED among women. Similarly, in the Beaver Dam Study of 3703 US adults, the age-adjusted prevalence of DED was significantly ~50% higher among women (16.7% among women versus 11.4% among men) [36]. In the Beaver Dam Offspring Study, which included younger adults, the prevalence of DED was also significantly higher among women (17.9%) as compared with men (10.5%) [37]. A discrepant finding emerged from the Salisbury Eye Evaluation of over 2400 US adults aged 65 and older, in which the

prevalence of one or more DED symptoms at least “often” was not significantly different at 15.6% among women versus 13.3% among men [38]. This result, combined with information from the Women’s Health Study and Physicians’ (men’s) Health Study, suggests the possibility that the sex difference in DED may lessen with more advanced age, becoming more similar among women and men. This possibility of effect modification by age could be evaluated in existing data as well as future studies.

European studies include estimates from the Alienor Study of 915 older French adults [39], reporting approximately 60% higher prevalence of self-reported DED and over two-fold higher reported use of artificial tears among women. In the Salnes study in Spain (N = 654) [40], the prevalence of at least one of six DED symptoms at least “often” was over 70% higher among women. However using a definition of at least one symptom plus one sign, the sex difference was diminished (11.9% among women versus 9.0% among men). Nonetheless, the prevalence of a tear breakup time of  $\leq 10$  s was also higher among women in this study (17.0% versus 12.8%).

Among the now numerous studies conducted in Asian countries, most but not all have reported significantly higher prevalence of DED among women (Table 2) [41–56]. For example, Hua et al. reported in a study of 2262 Chinese adults that women were significantly more likely than men to experience at least one DED symptom at least “often” [42]. Similarly, in a separate study of 1957 Chinese adults in the Beijing Eye Study, there was a significant 56% higher adjusted risk of at least one DED symptom at least “often” among women [43]. In contrast, Lu et al. in a study of over 1800 older Tibetans at high altitude, showed a similar prevalence of DED among women and men [44]. Overall, among 17 larger epidemiological studies in Asian countries, 11 showed a higher prevalence of symptomatic DED among women than men (ranging from 16% higher to nearly three-fold higher), 2 studies showed no difference in DED prevalence, and 2 studies showed a 43%–67% higher risk among males (Table 2) [41–56].

Overall, after review of large epidemiological studies of DED, the weight of the evidence supports a generally higher risk of DED among women. Reasons for observed differences across studies could include many factors, including the definition of DED, differences in characteristics of the populations studied (such as the age-distribution), and potential differences in risk factor profiles, health-seeking behavior and health service utilization.

### 2.2.2. Sex differences in quality of life indicators

In addition to a generally higher risk of DED among women, in a study of 1518 women and 581 men with diagnosed DED from the Women’s Health Study and Physicians’ (men’s) Health Study

respectively, Schaumberg et al. [57] observed that women were, on average, 6 years younger at the time of DED diagnosis (mean age at diagnosis = 60 years) compared with men (mean age at diagnosis = 66 years). In addition, women reported significantly higher levels of DED symptoms as measured by Ocular Surface Disease Index (OSDI) subscale and overall scores (each  $p < 0.0001$ ), as well as by The Symptom Assessment in Dry Eye questionnaire (SANDE) item and overall scores (each  $p < 0.0001$ ). Severe DED symptoms on the OSDI were reported by 33.6% of women compared with 15.6% of men, whereas 39.1% of women and 17.9% of men reported severe symptoms based on SANDE [57].

In the Women’s Health Study, women also reported a significantly greater impact of DED on visual quality indicators including blurred vision, poor vision, and fluctuating/unstable vision, as well as on tasks requiring sustained visual attention such as reading, driving at night, watching television, and working on a computer. Sex-related differences also extended beyond visual activities to greater feelings of depression among women, who were also less likely than men to report feeling calm and peaceful, or having a lot of energy.

### 2.2.3. Sex differences in burden of comorbidities

While there are now a large number of epidemiological studies that have reported on associated comorbidities [58], these studies generally did not report on the potential for sex-related differences in comorbidities. Among 3824 women from the TwinsUK cohort aged 20–87 years, the comorbid factors found to be most strongly associated with DED (highest effect sizes) included depression, pelvic pain, irritable bowel syndrome, and chronic widespread pain syndrome, and women with DED symptoms also scored significantly lower on self-perceived health [59]. In the Women’s Health Study, women who used postmenopausal hormone therapy were significantly more likely to have DED (~70% increased risk for estrogen alone, and ~30% for estrogen in combination with progesterone/progestins) [60]. In the all-male Physicians’ Health Study, reported DED-associated comorbidities included high blood pressure, benign prostatic hyperplasia and its medications, and use of antidepressants, and antihypertensives [35]. Further comparative analysis of women and men with diagnosed DED in the Women’s Health Study and Physicians’ (men’s) Health Study showed a significantly higher comorbid burden of lupus, Sjögren syndrome, rosacea, depression, anxiety, hay fever and dry mouth symptoms among women, whereas blepharitis and meibomian gland dysfunction were reported more frequently by men [61]. Women were also more likely to use antihistamines and antidepressants, but men were more likely to use glaucoma medications.

**Table 2**

Sex differences in the prevalence of DED in epidemiological studies in Asian populations.

Country	N	Definition	% higher risk in females
China [41]	1816	$\geq 1$ of 6 symptoms at least “often”	0%
China [42]	2262	$\geq 3$ of 7 symptoms at least “sometimes”	200%
China [43]	2009	$\geq 1$ of 6 symptoms at least “often”	56%
China [44]	1840	$\geq 1$ of 6 symptoms at least “often”	0%
Indonesia [45]	1058	$\geq 1$ of 6 symptoms at least “often”	43% greater in males than females
Japan [46]	598	Self-report based on symptoms	21%
Japan [47]	3433	Both dryness and irritation “constantly” or “often”	16%
Japan [48]	3549	Both dryness and irritation “constantly” or “often”	78%
Japan [49]	2644	Both dryness and irritation “constantly” or “often”	63%
Japan [50]	561	Japanese criteria	>200%
Singapore [51]	1004	$\geq 1$ of 5 symptoms at least “often”	64%
Singapore [52]	3280	$\geq 1$ of 6 symptoms at least “often”	67% higher in males
South Korea [53]	11,666	Dryness or irritation	93%
South Korea [54]	657	$\geq 1$ of 6 symptoms at least “often”	36%
South Korea [55]	16,431	Self-reported DED diagnosis	280%
Taiwan [56]	1361	$\geq 1$ of 6 symptoms at least “often”	30%

**Table 3**  
Sex-related differences in the anatomy, physiology and pathophysiology of the lacrimal gland, cornea, conjunctiva, meibomian gland, nasolacrimal duct and tear film.

Male	Female	Species
<b>Lacrimal gland</b>		
<ul style="list-style-type: none"> <li>• More frequent palpebral lobe periductal fibrosis &amp; focal atrophy in elderly</li> <li>• Higher median area of acini</li> <li>• Higher absolute weight</li> </ul>	<ul style="list-style-type: none"> <li>• Decrease in area &amp; thickness during aging</li> <li>• More frequent orbital lobe diffuse fibrosis, periductal fibrosis &amp; diffuse atrophy in elderly</li> <li>• Higher incidence of focal adenitis (especially in females &gt; 45 years old)</li> <li>• Higher prevalence of bilateral disease</li> <li>• Higher volume by MRI measurement</li> <li>• Smaller, more regular acini with narrow lumina</li> <li>• Acinar cell contours more conspicuous</li> <li>• Cell borders clear &amp; lobulated</li> <li>• Epithelial cells with clearer and somewhat structureless cytoplasm with heavy basophilic staining around nucleus (lighter toward periphery)</li> <li>• Basally-located nucleus showing more regularity in size &amp; shape</li> <li>• Many large cytoplasmic vesicles</li> <li>• Frequent intercellular channels</li> <li>• Capillary endothelia typically show pores</li> <li>• Higher incidence of glandular autoimmune disease</li> <li>• Higher acinar density</li> <li>• More severe fibrosis during aging</li> <li>• Increased number of intercalated, intralobular and interlobular ducts, especially in older animals</li> <li>• Higher collagen content in periacinar and periductal regions of older animals</li> <li>• Higher susceptibility to cytomegalovirus invasion and/or replication</li> <li>• Higher mast cell numbers</li> <li>• Greater expression of more than 1000 mRNAs (e.g. for androgen receptor, bcl-2, c-myc, c-myb, p53, IL-1<math>\beta</math> and TNF-<math>\alpha</math>, asialoglycoprotein receptor &amp; pancreatic lipase-related protein 1)</li> <li>• Multiple sex-specific responses in gene expression to hormone exposure</li> <li>• Higher synthesis of various proteins (e.g. melatonin, 20 kDa protein &amp; N-acetyltransferase, exocrine-gland-secreting peptide 36, lacrimal gland peroxidase, as well as leucine aminopeptidase after puberty)</li> <li>• Higher secretion of various proteins (e.g. 20 kDa &amp; 90 kDa proteins)</li> <li>• Greater amounts of melatonin &amp; N-acetyltransferase</li> <li>• Higher specific activity of Na<sup>+</sup>, K<sup>+</sup> -ATPase, cholinergic receptors, acid and alkaline phosphatase &amp; galactosyltransferase</li> </ul>	Human [14,192–198]
<ul style="list-style-type: none"> <li>• Large and irregular acini with wide lumina</li> <li>• Cell borders either indistinct or not apparent</li> <li>• Glandular epithelial cells with cloudy, light granular &amp; basophilic cytoplasm</li> <li>• Centrally located nucleus varying substantially in size &amp; shape</li> <li>• Marked nuclear polymorphism</li> <li>• Increased number of polyploid nuclei</li> <li>• Nuclei frequently harbor prominent nucleoli</li> <li>• Basal vacuoles and higher number of intranuclear inclusions in acinar cells</li> <li>• Sparse intercellular channels</li> <li>• Specialized structure of Golgi fields</li> <li>• Capillary endothelia display few pores</li> <li>• Enhanced labeling index of epithelial cells suggesting decreased cell turnover during aging</li> <li>• Increased acinar area during aging</li> <li>• More striking sexual dimorphism during aging</li> <li>• Greater extent of acinar metaplasia &amp; harderianization</li> <li>• Higher activity of hydroxyindole-o-methyltransferase &amp; carbonic anhydrase</li> <li>• Higher phenylephrine-induced secretion of peroxidase and total protein by gland <i>in vitro</i></li> <li>• Higher insulin-induced insulin receptor phosphorylation</li> <li>• Increased number of IgA-containing cells after puberty</li> <li>• Higher number of lymphocyte foci</li> <li>• Greater expression of more than 1000 mRNAs (e.g. for <math>\alpha</math>2 micro-globulin, secretory component [SC], cystatin-related protein, TGF-<math>\beta</math>1, Fas antigen, cysteine-rich secretory proteins-1 &amp; -3, mouse urinary protein, lipophilin AL2 &amp; androgen-binding protein subunits)</li> <li>• Multiple sex-specific responses in gene expression to hormone exposure</li> <li>• Higher production of various proteins (e.g. androgen receptor, immunoglobulin A [IgA] &amp; SC, lipocalin, exocrine-gland-secreting peptide 1, submandibular androgen-repressed protein)</li> <li>• Higher secretion of various proteins (e.g. SC, IgA, cystatin-related protein, 42 kDa &amp; 46 kDa proteins)</li> <li>• Higher number &amp; affinity of <math>\beta</math>-adrenergic binding sites</li> <li>• Greater total quantity of <math>\beta</math>-adrenergic receptors</li> <li>• Increased acinar cell lipid accumulation, and higher carbachol and pilocarpine-induced lacrimal gland secretion in the absence of P2X<sub>7</sub> receptor</li> </ul>		Mouse, rat, guinea pig, rabbit [11,34,64–109,199–205]
<b>Meibomian gland</b>		
<ul style="list-style-type: none"> <li>• Higher levels of 137 mRNAs (e.g. lysozyme, prolactin-induced protein)</li> <li>• Higher expression of specific polar lipids in meibum in both young and old men</li> <li>• Higher expression of specific neutral lipids in meibum in old men</li> <li>• Higher expression of specific fatty acid products, predominantly polar, in meibum than age-matched women</li> <li>• Higher casual level of meibum in young men</li> <li>• Higher incidence of abnormal lid margin and gland dropout in men <math>\geq</math>70 years-old</li> <li>• Greater decrease in symptoms following LipiFlow thermal pulsation treatment</li> <li>• Higher prevalence of asymptomatic MGD</li> </ul>	<ul style="list-style-type: none"> <li>• Higher levels of 120 mRNAs (e.g. S100 calcium-binding protein, leptin)</li> <li>• Higher expression of specific polar lipids in meibum in both young and old women</li> <li>• Higher expression of specific neutral lipids in meibum in old women</li> <li>• Higher expression of specific fatty acid products, predominantly polar, in meibum</li> <li>• Higher prevalence of meibomitis-related keratoconjunctivitis</li> </ul>	Human [7,10,110–116]

Table 3 (continued)

Male	Female	Species
<ul style="list-style-type: none"> <li>• Multiple sex-specific responses in gene expression to hormone exposure</li> <li>• Greater levels of more than 1000 mRNAs (e.g. for keratin 14)</li> <li>• Morphological appearance different than that of female glands</li> </ul>	<ul style="list-style-type: none"> <li>• Multiple sex-specific responses in gene expression to hormone exposure</li> <li>• Greater levels of more than 1000 mRNAs (e.g. for thyroid hormone responsive SPOT14 homolog)</li> <li>• Morphological appearance different than that of male glands</li> <li>• Greater size of meibomian glands in upper lid</li> </ul>	Mouse [6,8,90,117,118]
<b>Cornea</b>		
<ul style="list-style-type: none"> <li>• Thicker central, para-central and mid-peripheral zones of the epithelium</li> <li>• Male donor transplants: better survival rates</li> <li>• Higher horizontal diameter and sagittal height</li> <li>• More scarring in keratoconus</li> <li>• Higher levels of hundreds of mRNAs (e.g. epidermal growth factor receptor)</li> </ul>	<ul style="list-style-type: none"> <li>• Shorter wetting time</li> <li>• Better transplant recipient</li> <li>• Greater hysteresis and resistance factor</li> <li>• Higher sensitivity</li> <li>• Higher endothelial cell density</li> <li>• Higher prevalence of Salzmann nodular degeneration</li> <li>• Oxybuprocaine anesthetic eye drops induce significant corneal thickness increases only in females</li> <li>• Develop keratoconus at younger age</li> <li>• Fungal ulcers re-epithelialize twice as slowly</li> <li>• Higher levels of hundreds of mRNAs (e.g. transglutaminase 1)</li> <li>• Lower mean dry-eye severity score in premenopausal women following LASIK with femtosecond laser and mechanical keratome</li> <li>• Higher curvature</li> </ul>	Human [9,15,23,119–154]
<ul style="list-style-type: none"> <li>• Thicker central, para-central and mid-peripheral zones of the epithelium</li> <li>• Greater epithelial mitotic index rate</li> </ul>	<ul style="list-style-type: none"> <li>• Higher curvature</li> <li>• Slower corneal epithelial wound closure and lower polymorphonuclear leukocyte responses</li> </ul>	Mouse, dog, monkey [155–158]
<b>Conjunctiva</b>		
<ul style="list-style-type: none"> <li>• Higher goblet cell count</li> <li>• Higher prevalence of allergic conjunctivitis in boys</li> <li>• Higher prevalence of conjunctival inflammation in allergic rhinoconjunctivitis</li> <li>• Higher prevalence of pterygium</li> <li>• More distinct pinguecula</li> </ul>	<ul style="list-style-type: none"> <li>• Depression of goblet cell count around the time of ovulation</li> <li>• More discomfort from pterygia</li> <li>• Higher prevalence of superior limbic keratoconjunctivitis</li> </ul>	Human [159–166]
<b>Nasolacrimal duct</b>		
<ul style="list-style-type: none"> <li>• Greater length, diameter of the bony nasolacrimal canal entrance and volume</li> </ul>	<ul style="list-style-type: none"> <li>• More frequent appearance of mucopeptide, bacterial and mixed concretions (dacryoliths) in the lacrimal drainage system</li> <li>• Decreased aeration</li> <li>• Higher prevalence of primary acquired nasolacrimal duct obstruction</li> </ul>	Human [167–173]
<b>Tear film</b>		
<ul style="list-style-type: none"> <li>• Peak occurrence of short tear film breakup time in the third decade of life</li> <li>• Thicker and less contaminated lipid layer (&gt;45 years of age)</li> <li>• Higher amounts of EGF, TGF-<math>\alpha</math> and gender-specific tear protein</li> <li>• Higher tear osmolarity (&lt;41 years old)</li> <li>• Higher 30 kDa caseinolytic activity</li> <li>• Higher prevalence of dry eye symptoms in children (8.8 <math>\pm</math> 3 years old)</li> </ul>	<ul style="list-style-type: none"> <li>• Peak occurrence of short tear film breakup time in the seventh decade of life</li> <li>• Lower (non-invasive) tear breakup time, and an increase in tear osmolarity during aging</li> <li>• Higher amounts of innate immune defense proteins</li> <li>• Earlier decrease in tear peroxidase activity during aging</li> <li>• Higher prevalence of dry eye</li> <li>• Higher prevalence of dry eye disease after contact lens wear</li> <li>• Higher prevalence of dry eye after LASIK</li> <li>• Report of dry-eye symptoms more likely</li> <li>• Higher secretion and tear levels of various proteins (e.g. 20 kDa &amp; 90 kDa proteins)</li> </ul>	Human [18,121,150,174–189]
<ul style="list-style-type: none"> <li>• Higher tear levels of various proteins (e.g. SC, IgA, cystatin-related protein, TGF<math>\alpha</math>, 42 kDa &amp; 46 kDa proteins)</li> </ul>		Mouse, rat, hamster [100–104,190,191]

#### 2.2.4. Sex differences in DED treatment and treatment satisfaction

Schaumberg et al. [57] also described sex differences in treatment and treatment satisfaction. Women were significantly more likely than men to use traditional DED therapies such as artificial tears (82.8% versus 62.6%;  $p < 0.0001$ ), lubricating eye ointments (19.2% versus 11.7%;  $p = 0.0001$ ), and hot compresses (14.3% versus 10.7%;  $p = 0.02$ ). Among treatments categorized by the Management and Therapy Subcommittee of the TFOS DEWS report [62] as Level 2 or higher, women were also significantly more likely to use oral omega-3 supplements (18.6% versus 9.6%;  $p = 0.0006$ ), punctal plugs (15.0% versus 9.1%;  $p = 0.01$ ), and cyclosporine ophthalmic drops (13.4% versus 6.4%;  $p < 0.0001$ ). Although the majority of men and women expressed being at least “somewhat satisfied”

with DED treatments, there was a significant trend for higher dissatisfaction among women with the amount of time for treatment to work ( $p = 0.03$ ), and with treatment side-effects ( $p = 0.001$ ); these findings were potentially related to the higher proportion of women using topical cyclosporine, for which these downsides of therapy are widely recognized [57].

#### 2.2.5. Sex differences in the natural history of DED

In a subsample of 398 men and 386 women in the Physicians' (men's) Health Study and Women's Health Study who reported a diagnosis of DED and responded to a questionnaire about change, Leinert et al. [63] reported no significant differences by sex in reported worsening since the time of diagnosis (average 10.5 years) in

ocular surface symptoms, vision-related symptoms, or the social impact of DED. However, factors associated with worsening included a previous report of severe DED symptoms, which was highly correlated with female sex. Women also had a record of more frequent corneal staining/SPK on examinations and more frequent records of results of clinical tests for DED in their medical records [63].

Other aspects of the epidemiology of DED are covered in the TFOS DEWS II Epidemiology Report [58].

### 2.3. Sex related differences in the ocular surface and adnexa

Significant sex-related differences have been identified in the lacrimal gland, meibomian gland, cornea, conjunctiva, nasolacrimal duct and tear film. These differences, which may contribute, in part, to the female prevalence of DED, are listed in Table 3 [6–11], [14,15,18,23,34], [64–205] and are briefly summarized below.

#### 2.3.1. Lacrimal gland

Significant, sex-related differences exist in the anatomy, physiology and pathophysiology of the lacrimal gland (Table 3). Investigators have speculated that the increased diffuse atrophy, and orbital lobe and periductal fibrosis, present in the lacrimal glands of elderly women may decrease aqueous outflow and contribute to the sex-related prevalence of DED [193]. Additionally, the 50-fold greater expression of the asialoglycoprotein receptor (ASGPR) 1 gene in female mouse lacrimal tissue is particularly intriguing [83,89]. This receptor mediates the intracellular uptake of hepatitis C virus (HCV) [206], thereby promoting viral infection and exocrine gland inflammation [207,208]. In fact, chronic HCV infection may mimic the clinical manifestations of Sjögren syndrome [207–210] and is associated with an increased prevalence of keratoconjunctivitis sicca [211]. Another consideration is that the ASGPR is an autoantigenic target of both B- and T-cells [212]. If the ASGPR 1 gene is also upregulated in lacrimal glands of human females, and if the corresponding message is translated, this receptor expression could contribute to the increased prevalence of DED in women.

#### 2.3.2. Meibomian gland

Sex-related differences have been identified in the morphological appearance, gene expression, neutral and polar lipid profiles, and secretory output of the meibomian gland (Table 3). Of particular interest, the sex-related differences in gene expression across species are not necessarily the same. Comparison of the 100 genes with the greatest sex-associated differences in human (e.g. lysozyme, 18.2-fold, M > F) and mouse (e.g. androgen binding protein zeta, 109-fold, F > M) meibomian glands demonstrated that none of them were the same [111]. Similarly, whereas sex was found to exert a significant impact on numerous gene ontologies and KEGG pathways, these effects were also primarily species-specific [111]. The fact that sex differences are present in the meibomian gland might be expected, given that this tissue is a large sebaceous gland and sebaceous glands are known have sex-associated differences [213]. It is possible that these sex-related differences in the meibomian gland are a factor in the development of DED.

#### 2.3.3. Cornea

Significant sex-related differences exist in corneal anatomy and physiology (Table 3). Sex-specific changes in the cornea may also occur during the menstrual cycle, pregnancy and menopause. These alterations include variations in thickness, hydration, curvature and sensitivity, endothelial pigmentation, foreign body sensation, contact lens tolerance and visual acuity (Table 3) [214–222]. The sex-related variations in graft survival are very intriguing. While donor transplants from males have higher

survival rates than from females, transplants for female recipients show higher survival rates than for male recipients [132]. This fact raises the question whether the sex of the transplant must be considered in finding the optimal transplant for the recipient. Of particular relevance to DED is the finding that the human female cornea has a significantly greater expression of the gene for transglutaminase 1 (Table 3). This enzyme catalyzes protein cross-linking and its level is typically increased in DED and corneal keratinization [223,224].

### 2.3.4. Conjunctiva, nasolacrimal duct and tear film

Significant sex-related differences have been identified for example in the density of goblet cells and susceptibility to inflammation (Table 3). How these variations may relate to the sex-associated prevalence of DED is unclear. Nasolacrimal ducts of females are significantly shorter and narrower than those of men (Table 3). These features, as well as the acute angle between the bony canal and nasal floor in women, may predispose to chronic inflammation of the nasolacrimal drainage system and may explain why primary nasolacrimal duct obstruction is more frequent in females [173]. Significant sex-related differences exist in the tear film (Table 3). Overall, many of these sex-related differences in the ocular surface and adnexa are likely to be due to the influence of hormones and genetics.

### 2.4. Sex-related differences in immunity of the ocular surface and adnexa

#### 2.4.1. Sex and immunity

Sex differences in immune function affect both the innate and adaptive immune responses and manifest as differences in the prevalence and severity of infection and risk of developing autoimmune disorders [225,226]. In general infections are more common and more severe in males, females develop a greater antibody response and in some cases cell mediated response to vaccines but have a higher prevalence of many autoimmune diseases [225].

Examples of sex-specific differences in the innate immune response include: males have a greater percentage of pro-inflammatory cytokine producing monocytes than females [227]; females have less natural killer cell activity than males [228]; peripheral blood monocytes and plasmacytoid dendritic cells from females produce more IFN $\gamma$  upon stimulation than those from males [229,230]. Examples of sex-specific differences in the adaptive response include: females have greater numbers of CD4+ve T cells and greater CD4 to CD8 ratio than males [231] and show a preponderance towards a Th2 response whereas males predominantly generate a Th1 response; in keeping with the tendency towards a Th2 response women produce greater levels of circulating antibody than men [232] including higher levels of autoantibodies when affected by autoimmune diseases [233]; in women Treg numbers vary dramatically during the menstrual cycle with a high level in the follicular phase when estrogen levels are high [234]. As alluded to with the last example, the effects of steroid hormones estrogen, progesterone and testosterone can account, at least in part, for many of the sex-based differences in immune responses. Other contributors to the observed differences are genetics, microbiome and non-biological factors.

In terms of genetic effects an obvious point of discussion is the fundamental chromosomal make up of females being XX and males XY. The X chromosome has some 1100 genes (versus the Y which harbors less than 100) including several that are involved in immune function such as certain cytokine receptor subunits and Toll-like receptors, E26 transformation-specific domain-containing protein Elk-1 which is involved in B cell development and FOXP3 which is important for Treg development [225]. Early in

development one of the X chromosomes in a female is transcriptionally silenced in a random process such that in some cells it is the maternal X that is “turned off” and in others it is the paternal X. In this way mutations/polymorphisms in X-linked genes that may affect the immune response can be minimized in females whereas in males the effects of the altered gene will be manifest. In humans approximately 15% of the genes on the inactive X chromosome actually remain active thus it is also possible for females to have increased expression of some X-linked genes if both copies have remained active [235]. Depending on the gene involved this may contribute to enhanced immune responses. Interestingly, males with Klinefelter syndrome, where there is an additional copy of the X chromosome, show some immunological features, such as antibody levels, more similar to females than a normal XY male [236,237]. Further they have a 14-fold increase in the prevalence of lupus compared to XY males, with a similar risk for developing the disease as females [235].

Attention has been drawn to the potential role of epigenetic regulation by microRNAs (miRNAs) in sex-related immune differences. MicroRNAs are small double-stranded non-coding RNAs that negatively regulate gene expression by translational repression or mRNA destruction. Some 800 miRNAs have been identified in humans with approximately 10% being located on the X chromosome [238]. Several X-linked miRNAs are involved in immunity including miR-98 which regulates TLR mediated responses, miR-223 which negatively regulates granulocyte maturation and miR-503 and -542 which regulate monocyte differentiation [238,239]. It has been documented that miRNAs are differentially expressed among males and females in many tissues leading to the proposal that their differential expression in immune cells will also contribute to sex-related differences in immunity and susceptibility to autoimmunity [239]. Interestingly it was reported that several X-linked miRNAs were overexpressed in T cells from female lupus patients compared to males [240].

Another factor that may contribute to sex-differences in immunity are the microbial communities all humans harbor. Microbes, primarily in the intestine but also at other extra-intestinal niches, have been recognized to have an essential role in the development, maturation and modulation of the host immune response, [241]. Hormonal status can affect the composition of the microbiome in a sex specific manner while in turn members of the microbial community can metabolize sex hormones so influencing their effects on host immunity [242]. The existence of this relationship was shown in studies where manipulation of the microbiome conferred protection from autoimmune diabetes in female non-obese diabetic (NOD) mice in a sex hormone dependent manner [243,244]. Sex-related non-biological factors may also influence immunity [245]. For example some immunomodulators such as chemicals and metals produce work hazards that affect mostly males as they are the predominant sex working in that environment owing to the specific physical characteristics needed to perform the work, and also to gender norms for behavior.

#### 2.4.2. Influence of sex on ocular surface and adnexal immunity

The ocular surface and adnexa mount a very robust immune response to ensure the health of the eye and maintenance of good vision. The lacrimal gland contains a diverse array of T cells, B cells and their fully differentiated Ig secreting form – plasma cells, as well as dendritic cells and macrophages. The conjunctiva hosts secondary lymphoid tissue, called conjunctival associated lymphoid tissue and both cornea and conjunctiva are endowed with antigen presenting cells for rapidly responding to ocular surface antigens. Corneal and conjunctival epithelial cells also can respond to various antigens and synthesize and secrete many proteins that are important in innate and adaptive immune

responses [246,247].

Despite the long recognized sex-bias in general immunity (see Section 2.4.1) very few studies have examined sex-related differences in the ocular immune response *per se*. The tear film is an essential component of the ocular immune response and contains many components with antimicrobial functions [248] and a small number of studies have compared the levels of some of these antimicrobial components in males and females. Secretory IgA at the ocular surface binds and neutralizes pathogens and facilitates their removal. Although females produce higher levels of antibody than males [232] most studies have found no differences in the concentration of IgA in tears in humans [249–252]. A prominent sex-related difference has however been observed in rats where IgA and free secretory component were consistently higher in the tears of adult male rats than females [100,103]. Further, there was greater secretion of secretory component by the lacrimal tissue and a greater density of IgA positive cells in the lacrimal glands from male compared to female rats [75,102]. These effects appeared to relate to androgen function (see Section 3.1). Most studies show no sex-specific differences in tear levels of lactoferrin, lysozyme or phospholipase A2, all of which are antibacterial proteins [251–256]. In contrast, at least in rabbits, the concentration of lipocalin was increased in adult male rabbits in lacrimal fluid and lacrimal gland [98].

The density of corneal dendritic cells was not different in male and female contact lens wearers [257], although female mice on a C57BL/10 background lacking the capacity to produce  $\gamma\delta$  T cells had a much greater incidence of spontaneous keratitis than males [258]. These findings suggest that, at least in animals, some of the immune cells that act as a bridge between the innate and adaptive immune responses may be involved in sex-specific responses. In terms of the adaptive response, it has been observed that male and female rats have different patterns of T cell presence in the lacrimal gland over their lifetime [70,259]. Most studies have demonstrated that humans have no sex-related differences in lacrimal lymphocyte infiltration [195,259,260].

Thus, overall the preponderance of studies suggests few differences amongst the ocular surface/adnexal immune response in males and females in humans, at least in the absence of overt disease. However, it may be that the differences in immunity in males and females primarily manifest when the immune system is challenged. Thus, male sex has been found to be risk factor for developing keratitis with contact lens wear in some studies [261,262] (although the effect of gender behavior rather than sex might also explain this difference), and corneal re-epithelialization after development of fungal-related corneal ulcer took twice as long in females than males [149].

Sex-specific differences have been observed in some animal models of autoimmune DED, for example in the MRL/lpr mouse dacryoadenitis is significantly more severe in females than males whereas in NOD mice lacrimal gland pathology and lymphocyte infiltration was much greater in male than female mice [64,65,263]. How sex-related differences in immunity contribute to these observed variations has not been widely studied but recently it was observed that Treg dysfunction contributes to the male-bias seen in the NOD mice [264]. A study also addressed sex-specific differences in a desiccating stress model of DED [265]. Female mice exhibited more severe DED signs having increased corneal epithelial defects, decreased conjunctival goblet cells and lower production of mucin and tears compared to male mice. Interestingly the number of neutrophils in the lacrimal glands and draining lymph nodes of normal (non DED) mice was 2–4 times greater in females than in males. However, when experimental DED was induced in the animals the numbers of these neutrophils increased 2–12 fold in males but was decreased in females. Furthermore, the authors



found that in females, as the neutrophils decreased there were increases in Th1 and Th17 cells and a decrease in Treg cells, leading them to suggest the neutrophils were acting as suppressor cells, with neutrophil specific production of lipoxin A<sub>4</sub> mediating the effect [265].

### 2.5. Sex, gender, and the pain of DED

Pain is a difficult word to define, and many definitions have been proposed. “Whatever the experiencing person says it is, existing whenever s/he says it does” emphasizes the subjective experience of pain with no objective measures [266]. The latest and widely accepted definition of pain is “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”, which emphasizes that pain is a complex experience with multiple levels involved [267]. Detailed information related to the pathology of pain in DED is reported in the TFOS DEWS II Pain and Sensation Report [268], including pathways carrying pain signals to the brain, brain areas involved in pain perception, and the location, characterization and role of pain receptors in the lacrimal functional unit. Evidence in the literature on sex-related differences in pain is mostly not specific for the ocular surface. Relatively little attention has been paid to the relationships between sex and pain in DED as yet, but the current research on this issue is reported in this section.

Pain is common. The worldwide prevalence of chronic pain (defined as the pain that extends 3 or 6 months beyond onset or beyond the expected period of healing) is 25–30% [269–271], and is greatest in those affected by heart disease, cancer, and diabetes [272]. About a fifth of those who report chronic pain are thought to have predominantly neuropathic pain [269]. According to recent hypotheses, chronic pain in DED may also be a neuropathic pain [273,274]. It is known that female sex and older age are main factors associated with chronic pain [275]. The higher incidence of pain-related symptoms among women compared with men has been ascribed to sociocultural (gender-related) factors or biased reporting. However, sex differences in experimental pain response in animal or human studies and the higher prevalence in females of chronic pain syndromes [276] would suggest an underlying biological mechanism that is sex-related [277].

There are some common misconceptions about pain that have hindered the investigation of pain mechanisms and sex-related differences, in general and also specifically in DED. Some of the most popular incorrect beliefs include that i) pain does not exist in the absence of physical or behavioral signs or detectable tissue damage; ii) pain without an obvious physical cause is usually psychogenic; and iii) patients who respond to a placebo drug are malingering [266].

In DED, pain is difficult to describe due to the multifactorial nature and chronicity of the disease. Patients describe their sensations with a variety of expressions (dry/dryness, gritty, burn/burning hot, red, crust, shut, discomfort, visual changes, sore-irritated, gritty-scratchy, foreign body/foreign body sensation, burning, light sensitivity, itching, irritated, feeling of watery eyes, sharp, cutting, needle-like, pins and needles, pounding, pressure/aching) [18,274,278–281], so it may be difficult to correlate different descriptions with pain type and severity. Sex differences in reporting symptoms in DED have been found in a large epidemiological study [57]. Women with DED reported significantly greater problems with vision, reading, driving at night, watching television, and working on a computer compared to men with DED, as measured with OSDI subscales. This finding points primarily to perceived difficulties with visual tasks, but the tools for specifically measuring pain sensations (such as those described in 4.5.2) were not utilized in this survey.

A correlation also exists between DED and chronic pain as comorbidity [282,283]. Patients with non-ocular chronic pain diagnoses were more likely to carry a DED diagnosis compared with their counterparts without chronic pain. Fibromyalgia, a female prevalent disease, is now believed to be a brain disorder characterized by aberrant central pain facilitation and a state of hyperalgesia which may be due to impaired descending inhibition. Symptoms of fibromyalgia are often associated with DED symptoms [284], as are other conditions including chronic fatigue syndrome and sleep disorders.

#### 2.5.1. Sex differences in pain assessment

A reliable evaluation of a possible sex difference in subjective reporting of pain in DED remains lacking. Commonly utilized and rather easy to use, one-dimensional pain assessment tools include the Numeric Rating Scale (NRS), the Visual Analogue Scale (VAS), and the Faces Pain Scale (FPS) [285]. In DED literature these three scales have been described [286,287] yet seldom utilized in studies. In fact, the OSDI or other validated subjective symptom questionnaires are more frequently utilized, with the aim to score a sum of symptoms rather than pain intensity. To cover and quantify the multiple aspects of pain intensity and disability, multidimensional assessment tools are also used and include the Brief Pain Inventory (BPI) and the McGill Pain Questionnaire (MPQ), the latter focusing on assessment of sensory and affective dimensions of pain [285]. In DED literature, use of these tools, albeit limited, has just been reported [288].

#### 2.5.2. Sex differences in ocular surface sensitivity

As extensively reported in the TFOS DEWS II Pain and Sensation Report [268], functional types of corneal sensory receptors have been demonstrated and mapped [289]. Sex-related differences in ocular surface sensitivity are equivocal [140,290–292]. Premenopausal women have been found to be more sensitive to corneal stimulation than men of similar age, but overall there were no differences in mechanical and chemical thresholds between men and women [141]. High pain sensitivity and low pain tolerance, assessed with quantitative sensory testing using heat stimulus on the forearm, were found to be associated with symptoms of DED in female twin volunteers from the TwinsUK adult registry [293,294].

#### 2.5.3. Sex differences in general pain sensitivity, tolerance, intensity and unpleasantness

The current knowledge on chronic pain mechanisms involves complex brain circuits that include sensory, emotional, cognitive and interoceptive processing [295]. The neural networks join physiological systems (such as sensory, immune, endocrine, autonomic, motor systems and the sleep-wake rhythm) and psychological systems (such as perception, motivation, emotion, cognition, attention and memory) to behaviors [295]. It is difficult to draw firm conclusions as to sex influences in such complex interconnections. Various population-based studies suggested that women were more likely than men to experience a variety of chronic pain syndromes [296–299], and tend to report more severe pain [300], at a higher frequency and in a greater number of body regions [301]. However, results from reviewed literature were not always consistent and were affected by numerous confounding variables. Table 4 [302] presents a summary of data on the major outcomes from the literature for most pain modalities tested in multiple anatomical regions in the laboratory setting [297].

#### 2.5.4. Pain perception in DED subjects

As already reported in the TFOS DEWS II Pain and Sensation Report [268], experimentally induced pain perception in DED in humans can be assessed in the laboratory. Stimuli can be delivered

**Table 4**

Summary of data on the major outcomes from the literature for most pain modalities in the laboratory setting, from Racine et al., 2012a [302].

Type of stimulus	Pain threshold <sup>a</sup>	Pain tolerance <sup>a</sup>	Pain intensity or unpleasantness
Cold pain	W = M	W < M	No consistent difference
Hot pain	No consistent difference	W < M	No consistent difference
Pressure pain	W < M	W < M	No consistent difference
Ischemic pain	W = M	W = M	No consistent difference
Muscle pain	No consistent difference	No consistent difference	No consistent difference
Chemical pain	No consistent difference	No consistent difference	No consistent difference
Electrical pain	No consistent difference	No consistent difference	No consistent difference
Visceral pain	No consistent difference	No consistent difference	No consistent difference

<sup>a</sup> Pain threshold refers to the least experience of pain that can be identified by a subject; Pain tolerance is defined as the highest level of pain that a subject is able to tolerate.

to subjects by using 1. the controlled adverse environment (CAE) model [303–305], which induces DED signs and symptoms by regulating humidity, temperature, airflow, lighting conditions and visual tasking; 2. The Cochet-Bonnet esthesiometer, a handheld instrument in which a nylon filament is extruded, a calibrated scale provided by manufacturer is then used to convert filament length measurements to pressure. The instrument evaluates corneal and conjunctival sensitivity to mechanical pressure but has some limitations [306]; 3. The Belmonte Gas esthesiometer [307,308], and its modified version [309] which uses a jet of air to estimate ocular surface sensitivity to mechanical, chemical and thermal stimuli.

Sex was included as a variable playing a role in sensitivity thresholds in one study [308], where chemical sensitivity was found to be significantly lower in men than in women, whereas no significant differences were identified for the other stimuli. Another study [310] utilized the Cochet-Bonnet esthesiometer and found that corneal sensitivity was higher in men than in women, but only in superior, temporal and inferior areas. No sex-related differences were found in the corneal sensitivity response to cold stimuli induced by tear film evaporation during sustained eye opening in normal subjects or DED patients [311]. Conjunctival sensitivity, like that of the cornea, is higher in females than males with a trend towards an age-related increase in females, which is not apparent in males [140]. A study utilizing a different esthesiometer reported higher sensitivity of the cornea (and conjunctiva) in females compared to males, with an age-related increase apparent in females only [140].

#### 2.5.5. Sex differences in pain and the role of bio-psycho-social factors

Bio-psycho-social factors, include hormonal factors exposure to sex steroid hormones (biological factors), blood pressure, heart rate, peripheral and central processing of the stimuli (physiological factors), genotype, depression, anxiety/stress, coping, believing that something is worse than it is (psychological factors), and gender role expectations, experimenter sex/gender, past history of pain (social factors).

Extensive studies have investigated the role of endogenous or exogenously administered sex steroid hormones on pain sensitivity and perception. For a comprehensive review, readers are referred to Bartley and Fillingim [298]. Due to conceptual deficits such as small sample sizes, experimental session timing across the menstrual cycle and lack of biological markers to stage the cycle (such as urine or blood sample testing), the effects of estrogens on pain responses have been found to be inconsistent, minimal, or absent [298]. The role of androgen has been understudied [298]. Ocular discomfort (OSDI score) was higher during ovulation as compared to the luteal phase of the menstrual cycle [312,313]. Phytoestrogen or dehydroepiandrosterone (DHEA) supplementation was found to be associated with reduction in discomfort symptoms [314,315]. A weak correlation between higher levels of androstenedione and

subjective symptoms have been found [316]. (see also Section 3.1).

Physiological factors related to the nervous system and in particular to peripheral sensitization, primary hyperalgesia and central processing nociception, and allodynia are discussed in the TFOS DEWS II Pain and Sensation Report [268]. Here it was noted that methodologies employed to investigate the issue were not applied in DED in general or in sex-related differences specifically. Although phasic pupil dilation as a physiological marker of pre-conscious brain activity, brain activation imaging by positron emission tomography, and brain functional magnetic resonance imaging (fMRI) have failed to demonstrate clear sex differences in responses. There are clearly insufficient brain imaging data to draw firm conclusions that go beyond speculation, and more extensive research with positron emission tomography and fMRI is needed [298].

The role of genotype in pain is still understudied but some evidence is now emerging in terms of sex-related differences [276,317]. For instance, the melanocortin-1 receptor (MC1R) gene, associated with red hair and fair skin, has been found to moderate analgesia in a sex-dependent manner [317]. In DED, polymorphisms in the proinflammatory cytokine genes IL-1 $\beta$  (rs1143634) and IL-6R (rs8192284) were reported to be associated with non-Sjögren syndrome DED symptoms in a Korean population [318]. Chronic pain syndromes, including DED, have been reported to display a heritable component, as shown in a large twin-cohort population study [319] but a sex/gender-related difference was not shown.

#### 2.5.6. Sex differences in pain and the role of psychological factors

Inconsistent or contradictory results were obtained with regard to the direction of the association between anxiety/depression with sex and across outcome measures. Acute pain induces depressed mood [320] and chronic pain is known to cause depression [321]. Pain and depression are important comorbidities as both clinical and preclinical studies clearly indicate that pain is depressing, and depression can cause and intensify pain. But what comes first, and does a measure exist to objectively quantitate and time these events? [320,321] Sex differences in the prevalence of depression are widely established as depression is more common among females (21%) than males (13%) [322], although this might be confounded by gender differences in reporting depression or seeking treatment. Post-traumatic stress disorders are conditions that frequently coexist with chronic pain [323]. Post-traumatic stress disorders were more common in male veterans with DED than in those without and that male veterans with a DED diagnosis had a twofold higher risk of carrying a diagnosis of depression [324–326]. DED symptoms were more closely aligned to non-ocular pain, depression and post-traumatic stress disorders than to tear film parameters [327]. In female populations, depression, stress and DED symptoms were also closely correlated [59,328]. Of course no sex difference could be retrieved from any of these

papers.

The associations between depression, mood disorders, anxiety conditions and the severity of the symptoms in DED have been discussed in a case study [329]. The prevalence of sleep and mood disorders was found to be significantly higher in patients with DED and in correlation with age but not with sex [330]. In addition, depressive symptoms were found to be associated with DED symptoms [329,331–335] but no sex-related differences were reported in large population-based studies [336]. Taken from another perspective, a higher level of subjective happiness, as measured by a validated score [337], was inversely and significantly related to self-reported DED symptoms in a VDT users population but a sex-related difference was not found as well [338].

Other important psychological factors involve pain catastrophizing, a coping style which connotes negative emotional thoughts toward pain and adapting coping strategies [299]. In this respect, it is suggested that women tend to cope better with pain when they employ pain attentional focus or reinterpret pain sensation, whereas distraction may be more efficient in men [339–341]. None of these factors has been considered in DED papers as regards possible sex differences.

#### 2.5.7. Gender differences in pain and the role of social factors

Gender role broadly refers to a socially accepted set of characteristics ascribed to each sex. With regard to pain, the feminine role is stereotypically associated with greater willingness to report pain, whereas the expected masculine role is more related to stoicism. A measure of gender-related personality traits (masculinity–femininity) is given with the Bem Sex Role Inventory [342]. The emotional vulnerability related to the masculinity–femininity trait and the perceived identification according to typical male/female stereotypes seem to alter pain tolerance, intensity, and unpleasantness [343]. However, none of these social factors were considered in DED papers related to sex differences. Subjects perform better (i.e. higher pain tolerance or lower mean pain intensity) on a laboratory pain task when they are tested by an experimenter of the opposite sex [344]. Past history may influence pain perception in women but not in men. However, the literature needs to be enriched before drawing any strong conclusion. Finally, ethnic and cultural aspects represent an important but yet understudied issue [345,346]. None of these factors have been considered as regard

sex-differences in DED pain [347].

In conclusion, information on the relationship among DED, sex and the perception of pain is still not conclusive or unequivocal. Knowledge on possibly different sex-related mechanisms for pain process in DED is still limited. Sex differences in the response to experimentally induced ocular surface pain in healthy subjects remain understudied. Laboratory studies on sex-related differences in pain perception should be performed on healthy volunteers of various ages and on patients with painful pathologies (primary and secondary outcomes defined beforehand, sample size estimated as a function of clinical significance). The application of validated pain assessment tools in the clinic is still limited (only restricted to clinical trials) and standardized and more uniform testing procedures need to be adopted. The use of promising neuroimaging techniques is still very limited. All these points may represent the basis and suggestions for future studies.

### 3. Hormones and DED

The endocrine system plays a significant role in the regulation of, and the sex-related differences in, the ocular surface and adnexa. Hormones from this system are also implicated in the development and/or treatment of aqueous-deficient and evaporative DED. These hormones include androgens, estrogens, progestins, hypothalamic-pituitary hormones, glucocorticoids, insulin, insulin like growth factor 1 (IGF-1) and thyroid hormones. This section reviews the relevant actions of these hormones and their involvement in DED.

#### 3.1. Androgen regulation of the ocular surface and adnexa

Androgens are extremely important in the regulation of the ocular surface and adnexa [20,348–350]. They also appear to mediate many of the sex-related differences in these tissues [83,90,351–353]. Conversely, androgen deficiency is associated with both aqueous-deficient and evaporative DED [20,316,348–350,354–356]. In fact, as shown in an extensive metabolomics study of 390 different plasma metabolites in 1622 women with DED, unusual androgen metabolites (e.g. epiandrosterone, a hormone with weak androgenic activity) are key biomarkers for DED [357]. The impact of androgens on ocular surface and adnexal tissues is summarized below.

**Table 5**

Reported effects of orchietomy or androgen treatment on the lacrimal glands of mice, rats, hamsters, guinea pigs and/or rabbits.

Orchietomy	Androgen treatment
Glandular degeneration	Reversal of the effects of orchietomy on glandular structure, function & secretion
Decreased size of acinar cells & nuclei	Generation of many glycoprotein-secreting cells
Disappearance of vesicular mucus in acinar cells	Production of mucus & highly polymerized carbohydrates
Loss of cellular & nuclear polymorphism, & decreased nuclear volume	Appearance of PAS-positive material in acinar cells & central lumina
Fewer basophilic glandular cells & luminal enlargement	Induction of acinar cell & parenchymal hyperactivity
Alterations in levels of numerous mRNAs & proteins	Alteration in levels of thousands of mRNAs & numerous proteins
Attenuated alkaline phosphatase activity, & increase in N-acetyltransferase & hydroxyindole-o-methyl-transferase activity	Enlargement of glandular vesicles
Increased acinar epithelial cell susceptibility to cytomegalovirus infection	Reduction in total activity of cholinergic receptors
Proliferation of interfollicular connective tissue	Increase in total activity of Na <sup>+</sup> ,K <sup>+</sup> -ATPase, acid phosphatase, alkaline phosphatase & β-adrenergic receptors
Decreased or increased harderianization	Suppression of glandular inflammation
Alteration in fluid or specific protein secretion	Change in fluid or specific protein secretion
Transformation of structure to neutral (40 days after orchietomy) or female type morphology	Transformation of the glandular acino-serous structure into a “vesicular mucus” structure

Several of the responses listed in Table 5 are species- and/or strain-dependent [11–13,35,66,74,77–79,84,86–88,91,93,94,96,100,102–105,199,204,358–373,375–413]. In addition, older studies have reported no influence of orchietomy or androgen treatment on the growth or histological characteristics of the lacrimal gland [416,417]. These latter findings may be attributed in part to differences in experimental design, variations in the age, sex, and endocrine status of animals, the dosage and time course of androgen administration, and the methods of analysis.

### 3.1.1. Androgen regulation of the lacrimal gland

**3.1.1.1. Androgen influence and mechanism of action.** The lacrimal gland is an androgen target organ. Androgens exert a considerable impact on the structure and function of this tissue, including its cellular architecture, gene expression, protein synthesis, immune activity, and fluid and protein secretion [11,65,66,74,78,79,84,86,88,91,96,104,105,204,358–390]. Androgen deficiency, in turn, has been linked to lacrimal gland dysfunction and a corresponding aqueous tear deficiency [35,348–350,356,369,391–393].

Insight into the magnitude and extent of androgen influence on lacrimal tissue may be gained by reviewing the effects of orchietomy and/or androgen replacement therapy on this gland. As shown in Table 5 [11–13,35,66,74,77–79,84,86–88,91,93,94,96,100,102–105,199,204,358–373,375–413], investigators have reported that castration, or exposure to androgen receptor antagonists, significantly impair lacrimal gland anatomy and physiology. Alterations include degenerative changes, such as reduced growth and activity, loss of glandular elements, an attenuation in acinar cell size, a decrease in nuclear volume and polymorphism, proliferation of connective tissue, disruptions in protein levels, changes in enzyme activity, alterations in fluid and protein secretion, and a transformation of the gland's morphological appearance into a neutral or 'female' type. Conversely, researchers have also reported that androgen replacement therapy reverses the impact of castration, and may lead to profound changes in tissue structure, cellular activity and glandular secretion. These alterations may include acinar epithelial cell hyperactivity, the appearance of abundant glycoprotein secreting cells, an enlargement of glandular vesicles, the generation of mucus and highly polymerized carbohydrates, the suppression of inflammation, an evolution of the glandular acino-serous structure into a "vesicular mucus" pattern, and a change in tear protein and fluid secretion (Table 5).

Many of these androgen-induced effects have a molecular biological basis. As shown in studies with mice, androgens modulate the expression of thousands of lacrimal gland genes involved in biological processes, molecular functions and cellular components [90,351]. Gene ontologies most affected by testosterone include those associated with cell growth, proliferation and metabolism, cell communication and transport, nucleic acid binding, signal transduction and receptor activities [90,351]. Indeed, some of the most significant androgen actions are directed towards the stimulation of mitotic cycles, DNA metabolic processes and chromosomal components, and these responses may underlie androgen's ability to promote epithelial cell proliferation as seen in the rabbit [414,415], and a rise in tissue weight as seen in the mouse [358,384].

Numerous androgen derivatives influence lacrimal processes, such as the suppression of glandular inflammation in mouse models of Sjögren syndrome (see below example [66,91,97,100,102,103,204,367,372,374–377,380,412]). These hormones include: [a] testosterone derivatives (e.g. testosterone, 19-nortestosterone, methyltestosterone and fluoxymesterone); [b] 4, 5 $\alpha$ -dihydrotestosterone derivatives (e.g. dihydrotestosterone (DHT), methyl-dihydrotestosterone, oxymetholone, 5 $\alpha$ -androstano-17 $\alpha$ -ol-3-one-acetate, 5 $\alpha$ -androstano-17 $\beta$ -ol, stanozolol and 5 $\alpha$ -androstano-2 $\alpha$ -methyl-17 $\beta$ -ol-3-one); [c] 17 $\beta$ -hydroxy-5 $\alpha$ -androstano derivatives containing a ring A unsaturation, and excluding testosterone derivatives (e.g. 2, (5 $\alpha$ )-androstano-17 $\beta$ -ol); [d] 19-nortestosterone derivatives (i.e. 19-nortestosterone, 19-nortestosterone propionate); [e] 4-estren-7 $\alpha$ -methyl-17 $\beta$ -ol-3-one; [f] androgenic compounds with unusual structural features (i.e. oxandrolone and 5 $\alpha$ -androstano-17 $\beta$ -ol-3-oxime, which contains a nitrogen derivative substitution for the 3-ketone function in dihydrotestosterone); [g] adrenal cortical androgens (i.e. dehydroepiandrosterone, an androgen precursor, as well as expressing

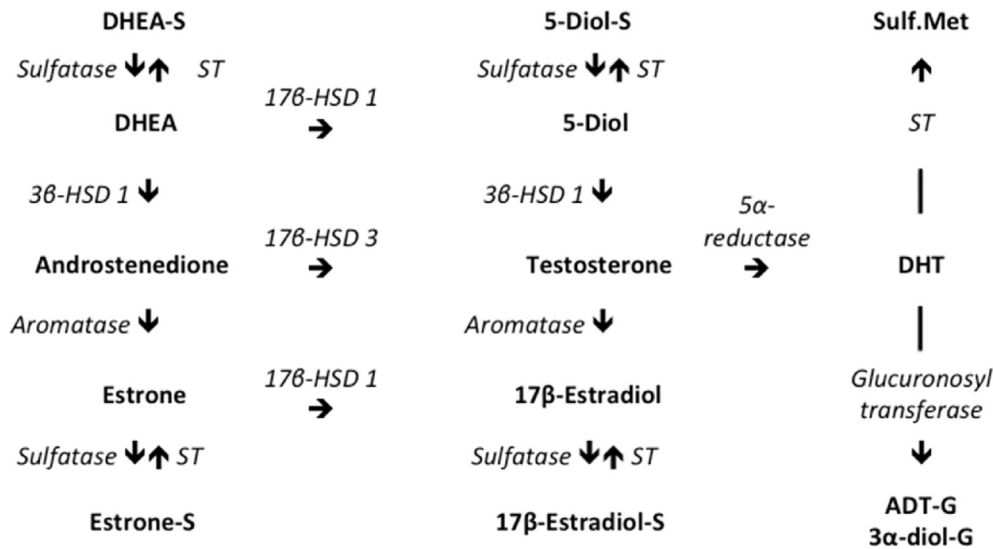
androgen action).

Androgen effects on the lacrimal gland may be enhanced or attenuated by a variety of neurotransmitters, cytokines, secretagogues, autoids, hormones, factors and viruses. Modulatory factors include vasoactive intestinal peptide,  $\beta$ -adrenergic agonists (e.g. isoproterenol), cholinergic agonists (e.g. carbachol), IL-1 $\alpha$ , IL-1 $\beta$ , tumor necrosis factor- $\alpha$ , cyclic AMP analogues (e.g. 8-bromoadenosine 3':5'-cyclic monophosphate), cyclic AMP inducers (e.g. cholera toxin and prostaglandin E2), phosphodiesterase inhibitors (e.g. 3-isobutyl-1-methylxanthine), pertussis toxin, insulin, glucocorticoids, cyproterone acetate, retinoic acid, prolactin, extracellular calcium, high-density lipoprotein, epidermal growth factor (EGF), fibroblast growth factor, putrescine, sialodacryoadenitis virus, cytomegalovirus, and factors from the pituitary, thyroid and adrenal glands [13,74,91,360,364,366,367,378,388,389,412,414].

The primary mechanism by which androgens act on the lacrimal gland appears to involve binding to saturable, high-affinity and steroid-specific receptors in acinar and ductal epithelial cells. These "classical" androgen receptors are members of the nuclear receptor superfamily of ligand-inducible transcription factors and appear to mediate most of the "classical" actions of androgens [418,419]. The location of androgen receptor protein is generally intranuclear, due to the presence of a nuclear targeting signal, similar to that of the SV 40 large T antigen, which occurs in the receptor hinge region immediately following the DNA-binding domain [420]. After androgen binding to the androgen receptor, the monomeric, activated hormone-receptor complex associates with androgen response elements in the regulatory region of specific target genes and, in combination with appropriate co-activators and enhancers, modulates gene transcription, protein synthesis and tissue function [418,419,421–424].

In support of this mechanism of action are the observations that: (a) androgen receptor mRNA is expressed in lacrimal glands of mice, rats, hamsters, guinea pigs, rabbits and humans [352,389,414,425,426]; (b) androgen receptor protein is present predominantly within epithelial cell nuclei of lacrimal tissues of mice, rats, hamsters and humans [97,372,385,389,427,428]; (c) lacrimal glands feature a single class of saturable, high-affinity and steroid-specific androgen binding sites, which have a dissociation constant and stereochemical selectivity similar to those found in many cells and tissues throughout the body [425,429]; (d) androgen-androgen receptor complexes in lacrimal tissue associate with DNA [429]; (e) androgen effects in lacrimal glands or in isolated acinar epithelial cells may be curbed by antagonists of, or mutations within, androgen receptors, as well as by inhibitors of transcription and translation [88,102,199,367,414]; (f) androgens, as noted above, exert a significant influence on gene expression and protein synthesis in lacrimal glands [11,13,74,77,79,86–88,91,97,100,102,104,105,352,353,359,363,364,366,371–373,378,381–383,386,388,389]. Androgens also modulate the expression of their own androgen receptors in the lacrimal gland by increasing the content of androgen receptor protein and decreasing the level of androgen receptor mRNA [97,372,389,430]. This form of autoregulation also occurs in other, but not all, androgen target organs [431–435].

In addition to nuclear androgen receptors, androgens may possibly act on the lacrimal gland through nonclassical pathways. These pathways, which typically occur within seconds to minutes, involve hormonal interaction with stereospecific plasma membrane receptors and lead to rapid changes in membrane fluidity, the activity of neurotransmitter receptors and/or the control of transcription factors [424,436–442]. However, evidence for such lacrimal membrane receptors has yet to be obtained. Androgen-binding proteins have also been identified in lacrimal glands and tears of male and female mice, but not yet examined in humans [443–445]. The role of androgen-binding proteins is unknown.



**Fig. 1.** Major metabolic pathways of androgens and estrogens in humans. The directions of enzymatic reactions are shown by arrows. Abbreviations are 17β-HSD 1, 17β-HSD 3 = 17β-hydroxysteroid dehydrogenases types 1 or 3; Sulfatase = steroid sulfatase; 3β-HSD 1 = 3β-hydroxysteroid dehydrogenase (HSD)- $\Delta^5\Delta^4$ -isomerase type 1; ST = sulfotransferase; Sulf.Met = sulfated metabolites; HSD = hydroxysteroid dehydrogenase; DHEA = dehydroepiandrosterone; DHEA-S = DHEA sulfate; Estrone S = estrone sulfate; DHT = dihydrotestosterone; 5-diol = 5-androstene-3β,17β-diol; ADT-G = androsterone-glucuronide; 3α-diol-G = androstane-3α,17β-diol-glucuronide. Modified and reprinted with permission from Wolters Kluwer Health, Inc [459].

The source of androgens that act on the human lacrimal gland may primarily be from local, intracrine synthesis (Fig. 1). As demonstrated in the field of intracrinology, the vast majority of androgens in women (i.e. most before and all after menopause), and a significant percentage in men (e.g. 40–50%), are synthesized in peripheral tissues from adrenal sex steroid precursors (i.e. DHEA, DHEA-sulfate [DHEA-S] and androstenedione) [446–458]. In fact, humans and primates are unique in possessing adrenal glands that secrete large amounts of DHEA and DHEA-S, which are then converted into androgens (e.g. testosterone, DHT) and estrogens by steroidogenic enzymes in peripheral sites and allow target tissues to adjust the formation and metabolism of sex steroids to local requirements [449,453]. Human lacrimal glands produce mRNA for Type 1 and 2 5α-reductase [427], and mRNAs for steroid sulfatase, 3β-hydroxysteroid dehydrogenase (HSD)- $\Delta^5\Delta^4$ -isomerase type 1, 17β-hydroxysteroid dehydrogenase types 1 and 3, aromatase, glucuronosyltransferase and sulfotransferase [459].

The presence of aromatase in the lacrimal gland raises the question as to whether many of the androgen actions on the lacrimal gland might possibly be mediated through its aromatization to estrogens. Aromatase is an enzyme that catalyzes the transformation of testosterone and androstenedione to 17β-estradiol and estrone, respectively. The answer is no. Less than 2% of the genes upregulated by testosterone in lacrimal tissues of orchietomized and ovariectomized mice are also increased by 17β-estradiol [90]. Moreover, of those genes influenced by both testosterone and 17β-estradiol in lacrimal glands of castrated female mice, over 60% of the sex steroid effects are in the opposite direction [90]. Thus, androgen action on the lacrimal gland is not mediated primarily through a conversion to estrogens.

The effects of androgens on lacrimal gland structure are not the same in all species [204]. For example, androgen administration increases the acinar epithelial cell area and lacrimal gland weight/body weight (LGW/BW) ratio in intact female mice [358,374,376,384], but rarely alters these variables in lacrimal glands of castrated male or female rats or rabbits, and may even reduce the LGW/BW ratio in guinea pigs [204]. Further, androgens promote the proliferation of rabbit acinar epithelial cells *in vitro*, [414,415] but do not induce such an effect on rat lacrimal gland cells

in culture [364]. Consequently, although androgen action on lacrimal gland structure is considerable, it is unlike that of the ventral prostate, which in most species is completely dependent upon androgens for size maintenance and undergoes involution and programmed cell death following androgen withdrawal [460].

The impact of androgens on lacrimal gland secretion is also not the same in all species. Although androgens regulate the lacrimal gland output of certain proteins [13,98,100,102,103,105,377,412,413], these hormones do not elicit a consistent, species-independent action on fluid or total protein secretion [204]. Instead, androgens induce time-, strain- and species-dependent effects, leading to a non-uniform increase, decrease or no impact on the volume and total protein level of tears in mice, rats, guinea pigs, rabbits and humans [12,204,361,369,376,377,384,387,393,396,397,408,461–464]. Of particular interest is the observation that androgens upregulate the expression of cystatin-related proteins in the rat lacrimal gland [88,105]. Cystatin 4 (also called cystatin S), in turn, is one of the main discriminant protein biomarkers in the tear film for differentiating between people with and without DED [465,466].

### 3.1.1.2. Clinical relevance of androgen influence on the lacrimal gland

**3.1.1.2.1. Sjögren syndrome.** Androgen deficiency appears to be a risk factor for, but not a cause of, the development of lacrimal gland inflammation and aqueous-deficient DED in women with Sjögren syndrome. Women with Sjögren syndrome are androgen-deficient [391,467–472]. The serum levels of DHEA, 5-androstene-3β,17β-diol, DHT, androsterone-glucuronide (ADT-G) and androstane-3α,17β-diol -glucuronide (3α-diol-G) are significantly reduced in women with this autoimmune disorder [391]. The decrease in ADT-G and 3α-diol-G concentrations is noteworthy, because these glucuronidated DHT metabolites reflect the total intracrine production and metabolism of androgens in peripheral tissues and appear to be the most valid and reliable measures of the total androgen pool in humans [447,451,452].

An even greater androgen deficiency may exist in lacrimal tissues of Sjögren syndrome patients. The reason is that levels of proinflammatory cytokines, such as IL-1, TNF-α and IL-6 are elevated in exocrine tissues in Sjögren syndrome [85,373,430,

473–479]. These cytokines may disrupt the normal activity of steroidogenic enzymes and promote the aromatization of testosterone to 17 $\beta$ -estradiol [480–484]. These cytokines may also attenuate the expression of androgen receptor mRNA [485], interfere with certain androgen actions [486] and stimulate corticosteroidogenesis, which potentiates the aromatization of androgens, resulting in decreased testosterone and increased estrogen levels [487]. This decrease in testosterone would enhance inflammation, given that androgens typically suppress the expression of TNF- $\alpha$ , IL-1 $\beta$  and IL-6 and potentiate the levels of the anti-inflammatory cytokine IL-10 [464,488–490]. In contrast, the increase in estrogen might also enhance inflammation, because this hormone may stimulate TNF- $\alpha$ , IL-1 $\beta$  and IL-6 production, synergize with IL-1 $\beta$ , and attenuate IL-10 amounts [488,491,492]. This androgen deficiency would compromise the positive regulatory influence of androgens in lacrimal tissues of Sjögren syndrome patients, and predispose to the development of glandular dysfunction, inflammation, reduced tear secretion and aqueous-deficient DED. Conversely, correcting this androgen deficit in Sjögren syndrome may have a therapeutic effect on the lacrimal gland.

In support of these deductions are the following observations. Testosterone treatment of female mouse models of Sjögren syndrome (i.e. MRL/Mp-lpr/lpr [MRL/lpr] and NZB/NZW F1 [F1]) causes a dramatic suppression of the inflammation in, and a significant increase in the function of, the lacrimal gland [348,358,374–377,384]. This hormone action may be duplicated by administering a number of “anabolic” or “androgenic” androgen analogues, but not by exposure to estradiol, danazol, cyclosporine A (CsA), dexamethasone or experimental non-androgenic steroids [13,374]. In addition, this anti-inflammatory effect seems to be site-specific: androgens decrease lymphocyte accumulation in lacrimal, as well as salivary, glands, but do not diminish the extent of inflammation in lymphatic and splenic tissues [376], or lessen certain systemic immune dyscrasias [493–495]. 19-nortestosterone administration to the ocular surface completely resolves the inflammation in nictitating lacrimal glands of dogs with DED [496,497]. Topical or systemic androgen administration significantly decreases DED signs and symptoms, and stimulates tear flow, in patients with Sjögren syndrome [394,396,397,461]. These findings support the hypothesis that androgen deficiency is a significant risk factor in the pathogenesis of lacrimal gland dysfunction, inflammation and aqueous-deficient DED in Sjögren syndrome.

That such an androgen-autoimmune interrelationship may exist is not surprising. Researchers have shown that androgen insufficiency may contribute not only to the prevalence of Sjögren syndrome, but also to the sex-specific expression of other autoimmune diseases. This sexual dichotomy, in turn, has been linked to the differential actions of sex steroid hormones on the immune system [487,498,499]. Estrogens often enhance, whereas androgens frequently attenuate, the progression of autoimmune sequelae [348,379,394,498–508]. In effect, androgen deficiency appears to promote the development of certain autoimmune states. This may explain why androgen therapy has been successful in alleviating various signs and symptoms in animal models of systemic lupus erythematosus, thyroiditis, polyarthritis, autoimmune hemolytic anemia, and myasthenia gravis, as well as in humans with rheumatoid arthritis and systemic lupus erythematosus [348,394,396,397,487,498,499,505–508].

The mechanism(s) by which androgens suppress lacrimal gland inflammation in Sjögren syndrome has yet to be clarified. One process, though, likely involves a hormone-induced decrease in inflammatory gene activity. For example, androgens suppress the lacrimal gland mRNA levels of caspase 1 [351], which is known to activate the pro-inflammatory cytokines IL-1 $\beta$  and IL-18, as well as

promoting the efficient expression of IL-1 $\alpha$  [509]. Androgens also reduce the lacrimal gland expression of MHC Class II antigen processing genes, as well as that for ASGPR 1, which has been implicated in the development of exocrine gland inflammation [207,208,211]. In autoimmune MRL/lpr mice, testosterone has been shown to significantly decrease the expression of genes related to inflammatory responses, immune cell chemotaxis and antigen presentation [510]. These androgen actions appear to be initiated through androgen binding to non-defective androgen receptors in lacrimal gland epithelial cells [372,511]. Acinar and/or ductal epithelial cells, in turn, are thought to be the primary cells involved in the initiation and perpetuation of autoimmune reactivity in Sjögren syndrome [512]. This androgen-epithelial cell interaction may then induce the altered activity of specific genes and proteins in lacrimal tissue (e.g. cytokines, proto-oncogenes and apoptotic factors), leading to the reduction of immunopathological lesions and an improvement in glandular function [348,358,374,375,377,384].

In contrast to the above, some investigators have proposed that androgen insufficiency causes an autoimmune process in the lacrimal gland, leading to inflammation, a Sjögren syndrome-like pathology and aqueous tear deficiency [361,362,513,514]. In support of this hypothesis, researchers have reported that androgen withdrawal triggers glandular atrophy, involving reduced acinar size, acinar cell necrosis and extensive regions of acinar cell degeneration [361,362,513]. These alterations are proposed to stimulate the generation of autoantigens, the development of lacrimal gland autoimmune disease and the induction of a Sjögren syndrome-like aqueous tear insufficiency [361,362,514]. Androgen administration, in turn, may prevent lacrimal gland regression [361,362,387], thereby suggesting that these hormones are essential for maintaining fluid secretion by lacrimal tissue.

However, these hypotheses are not supported by other studies. Lacrimal glands from young and old testicular feminized (*Tfm*) mice do not show any histological evidence of inflammation due to androgen deficiency [353]. These *Tfm* mice, which are often used to evaluate androgen-dependent phenomena, possess dysfunctional androgen receptors and are resistant to androgen influence [515]. Moreover, androgen synthesis in *Tfm* mice is severely reduced [516]. This insufficiency, when coupled with the androgen receptor defect, would serve to prevent both classical and nonclassical effects of androgens. Consequently, if androgen deficiency causes lacrimal gland inflammation, one might anticipate that this effect should be apparent in *Tfm* mice. Such inflammation, though, is not present [353]. Similarly, investigators have found that androgen deficiency caused by castration and/or interruption of the hypothalamic-pituitary axis does not induce any lymphocyte accumulation in, or regression of, the lacrimal glands in male and female rats, guinea pigs and/or rabbits [204,353]. Researchers have also demonstrated that androgen receptor dysfunction (e.g. *Tfm* mice) and androgen insufficiency (e.g. men taking anti-androgen medications) do not cause aqueous tear deficiency [353]. Lastly, complete androgen receptor absence induces premature ovarian failure [517,518], a condition in humans that causes a non-aqueous-deficient DED [519]. Overall, it appears that androgen deficiency promotes, but does not cause, the lacrimal gland inflammation in Sjögren syndrome. Further, androgen deficiency may impair lacrimal gland function, but does not seem to induce aqueous-deficient DED.

Lastly, it should be noted that androgens induce lymphocyte accumulation in the lacrimal glands of NOD mice [264,520]. These NOD mice have been proposed as models of Sjögren syndrome and develop lacrimal gland immunopathology [521–523]. However, in contrast to the situation in humans, as well as in MRL/lpr and F1 mice, it is the male, and not female, NOD mice that feature

extensive autoimmune disease in their lacrimal gland [64,65,263]. This anomalous effect is mediated through the lacrimal microenvironment [64] and male-specific factors that cause CD4(+) CD25(+) Foxp3(+) regulatory T cell dysfunction [264], and contrasts with the androgen-induced suppression of inflammation in NOD salivary and pancreatic tissues [64,524,525].

**3.1.1.2.2. Non-autoimmune disease.** Researchers have reported that low serum concentrations of testosterone are also more prevalent in women with DED and correlate with the subjective severity of ocular symptoms [526]. However, the reason for this association is unclear, given that serum testosterone levels reflect only a very small fraction of the total androgen pool in women [446–458]. In fact, investigators have proposed that the measurement of serum testosterone in women may have little or no value except as an index of ovarian activity [447,449,451]. Intracrine synthesis in peripheral tissues, and not the ovary, is the primary source of androgens (or estrogens) in human females [447,449,451,453,455–457].

Researchers have also suggested that the decrease in serum androgen levels that occurs during menopause, pregnancy, lactation, or the use of estrogen-containing oral contraceptives may trigger the development of a non-immune type of DED, termed *primary lacrimal gland deficiency*. [387] Others have reported that ovariectomy, with [527] or without [528] Type II 5 $\alpha$ -reductase inhibitor (i.e. finasteride) treatment, will cause aqueous tear deficiency – apparently due to a loss of androgens. If these suggestions and/or reports are correct, then they may represent a response that is sex-specific (i.e. females, not males). The reason is that extended (e.g. years) exposure to anti-androgen therapy for prostatic disease has no effect on the aqueous tear production (i.e. Schirmer test), as compared to that of age-matched, untreated controls [353]. Consequently, androgen deficiency in males who show no evidence of immune pathology (e.g. autoimmune disease) does not appear to promote the development of aqueous tear deficiency.

**3.1.1.2.3. Secretory immunity.** In experimental animals, androgens have been demonstrated to stimulate the lacrimal gland secretory immune system, which helps protect the anterior surface of the eye against microbial infection and toxic challenge [529–532]. This immune function is mediated primarily through secretory IgA (sIgA), which originates from plasma cells in the lacrimal gland [533,534], is transported across epithelial cells into tears by the polymeric Ig receptor (i.e. secretory component [SC]) [13] and acts to prevent viral invasion, inhibit bacterial colonization, interfere with parasitic infestation and suppress antigen-induced damage on the ocular surface [529–532]. In rats, androgens induce the synthesis and secretion of SC by lacrimal gland acinar epithelial cells, increase the concentration of IgA in lacrimal tissue and promote the transfer and accumulation of SC and IgA in tears [535]. Androgens also stimulate the lacrimal gland output of IgA, presumably through SC regulation, into tears of mice [377]. Considering that the promoter region of the human SC gene contains several putative androgen receptor binding sites [536], it may be that androgens also enhance SC production in the human lacrimal gland. Such an androgen-induced increase of SC synthesis was demonstrated in human lacrimal gland epithelial cells [537]. Thus, androgens may stimulate sIgA transport into human tears and thereby promote the maintenance of corneal and conjunctival integrity and the preservation of visual acuity. Further, androgen deficiency could possibly be a factor in the decreased levels of IgA in tears of DED patients [538], and especially those of patients with Sjögren syndrome [539].

### 3.1.2. Androgen regulation of the meibomian gland

**3.1.2.1. Androgen influence and mechanism of action.** The meibomian gland, a large sebaceous gland, is an androgen target organ.

Androgens stimulate this tissue's function and appear to suppress its keratinization [2,10,462,540–544]. Androgen deficiency, in turn, is a risk factor for meibomian gland dysfunction and a corresponding evaporative DED [2,10,35,391,392,540–542].

Androgens are known to modulate the development, differentiation and lipogenesis of sebaceous glands [545–549]. Sebaceous gland activity and secretion, in turn, may be antagonized by orchiectomy or topical anti-androgen treatment [550–554]. Androgen actions on the meibomian gland appear to be mediated, at least in part, through binding to classical nuclear receptors. The meibomian glands of male and female rats, rabbits and humans contain androgen receptor mRNA and androgen receptor protein within acinar epithelial cell nuclei [426,427,543]. In addition, androgens regulate the expression of numerous genes in mouse, rabbit and human meibomian glands [547,555–560]. These effects appear to depend on the presence of functional androgen receptors [556,557]. However, whether all androgen effects on the meibomian gland are mediated through classical receptors remains to be clarified. It is possible that androgen action may also involve binding to membrane receptors, triggering of signal transduction cascades and associated changes in gene transcription [561,562].

Androgen activity in the human meibomian gland may occur primarily after local synthesis from adrenal precursors. Human meibomian glands contain the mRNAs for all the enzymatic machinery necessary for the intracrine synthesis and metabolism of androgens, including steroid sulfatase, 3 $\beta$ -HSD)- $\Delta^5\Delta^4$ -isomerase type 1, 17 $\beta$ -HSD types 1 and 3, Types 1 and 2 5 $\alpha$ -reductase, aromatase, glucuronosyl-transferase and sulfotransferase [427,459]. Further, 3 $\alpha$ -HSD, 3 $\beta$ -HSD and 17 $\beta$ -HSD, at least, are translated in epithelial cells of the human meibomian gland [563].

Androgens exert a significant impact on gene expression in the human meibomian gland. For example, DHT regulates the expression of almost 3000 genes in immortalized human meibomian gland epithelial cells (IHMGEs) [560]. Most striking are the effects of DHT on lipid- and keratin-related genes. Androgens stimulate 25 different ontologies (with  $\geq 5$  genes) concerned with lipid biosynthesis, homeostasis, transport and binding, as well as with cholesterol, fatty acid, phospholipid and steroid dynamics [110]. This hormone response is similar to the androgen influence on mouse meibomian glands *in vivo* [90,547,558,559] wherein testosterone upregulates many genes linked to lipid metabolic pathways. DHT administration also led to a 40-fold decrease in the mRNA level of small proline-rich protein 2A (SPPR2A) in IHMGECs. The expression of this SPPR2A gene is significantly upregulated in human MGD [110], and it encodes a protein that promotes keratinization [564]. Keratinization, in turn, is a primary cause of MGD and so may contribute to tear film hyperosmolarity and evaporative DED [20]. The SPPR2A gene, as well as other genes related to keratinization, is also significantly downregulated by testosterone in meibomian glands of male and female mice [90,547]. These combined androgen effects, increasing lipogenesis and suppressing keratinization, may begin to explain how topical androgens increase the synthesis and secretion of meibomian gland lipids, prolong tear film breakup time and alleviate evaporative DED in humans (see below) [462,544]. In addition, these DHT effects on IHMGECs may account for why androgen deficiency (e.g. during anti-androgen treatment, complete androgen insensitivity syndrome [CAIS] and/or aging) is associated with altered meibum lipid profiles, a reduced quality of meibomian gland secretions, and keratinization of the meibomian gland ductal epithelium (i.e. orifice metaplasia) in humans [10,112,540–542].

Androgen treatment also causes a significant change in the expression of many other genes in IHMGECs, such as those associated with steroidogenesis, microbial protection, tissue development, oxidative stress, mTOR (mechanistic target of rapamycin) and

PPAR (peroxisome proliferator-activated receptor) signaling, cell cycle, innate immunity and angiogenesis. DHT upregulates the mRNA levels of defensin  $\beta$ 1, an antimicrobial peptide implicated in epithelial surface resistance to microbial colonization [565], as well as steroid-5 $\alpha$ -reductase,  $\alpha$  polypeptide 1, which catalyzes the conversion of testosterone into the more potent androgen, DHT [565]. This steroid effect appears to be a form of feed-forward control exerted by DHT on its own biosynthesis [566]. DHT increases the gene expression of leptin receptor, involved in the regulation of fat metabolism, glucose homeostasis, wound healing and the immune system [565], as well as FOXO1 (forkhead box protein O1), a transcription factor that mediates cell responses to oxidative stress [565] and is known to interact with androgen receptors [567], and stearoyl-CoA desaturase, an iron-containing enzyme that catalyzes the synthesis of unsaturated fatty acids. Similarly, testosterone increases stearoyl-CoA desaturase mRNA levels in male and female mouse meibomian glands [90,547], and the targeted disruption of this rate-limiting enzyme results in meibomian gland atrophy [568]. DHT also stimulates ontologies and pathways in IHMGECs related to peroxisomes, which are organelles involved in metabolism of fatty acids and other metabolites [565]. DHT stimulates PPAR, which may promote tissue differentiation [569,570] and mTOR, a serine/threonine protein kinase that may regulate cell growth, cell proliferation, cell motility, cell survival, protein synthesis and transcription [565,571,572], which is also activated by androgens in the prostate [573]. DHT downregulates genes in IHMGECs associated with cell cycle regulation, innate immunity and angiogenesis. Androgen exposure also suppresses the IHMGEC gene expression for matrix metalloproteinase 9, an enzyme elevated in the tear film in DED and known to promote corneal inflammation [574], as well as the lipopolysaccharide (LPS)-induced secretion of leukotriene B4 (LTB4), a pro-inflammatory mediator [575].

In animal studies testosterone has been shown to regulate the expression of over 1000 genes in the meibomian glands of male and female mice [90,547,555,557–559]. Many of the upregulated genes are associated with lipid metabolism (e.g. sterol regulatory element binding protein), lipid transport (e.g. lipocalin 3), sterol biosynthesis (e.g. 3-hydroxy-3-methylglutaryl CoA-reductase) and fatty acid metabolism (e.g. fatty acid synthase), as well as with intracellular protein transport, oxidoreductase activity, peroxisomes, mitochondria and early endosomes [90,547,555,557–559]. Testosterone also increases the activity of a series of genes that may be very important in the endocrine control of the meibomian gland [547]. Testosterone stimulates activity of 17 $\beta$ -HSD 7, an enzyme that catalyzes the interconversion of 17-ketosteroids with their corresponding 17 $\beta$ -hydroxysteroids [576]. This enzyme is critical for the metabolism of all active androgens and estrogens [576] and may mediate the local, intracrine synthesis of androgens from adrenal precursors in the meibomian gland. Testosterone stimulates insulin-like growth factor 1, a pleiotropic protein that stimulates sebocyte proliferation, differentiation and signaling [577]. Estrogen receptor (ER)  $\beta$ , a receptor that is upregulated by androgen in the prostate [578] and may antagonize the activity of ER $\alpha$  [579] is stimulated by testosterone, as is 11 $\beta$ -HSD 1, an enzyme that converts cortisol to the inactive metabolite, cortisone.

Androgens also influence the lipid, and possibly protein, composition within the meibomian gland. Orchiectomy causes a marked alteration in the lipid profile of rabbit meibomian glands, whereas the topical or systemic administration of 19-nortestosterone, but not placebo compounds, for 2 weeks begins to restore the lipid pattern towards that found in intact animals [543]. In addition, investigators have speculated that androgen-signaling machinery regulates the expression of secretoglobulin in human meibomian gland epithelial cells [580]. This protein may be released and possess a lipocalin-like function in the tear film

[466,580].

3.1.2.2. *Clinical relevance of androgen influence on the meibomian gland.* Androgen deficiency is a risk factor for the development of MGD [2,20]. Such hormone insufficiency typically occurs during menopause (decrease in ovarian and adrenal 'androgen' secretion) [355,447,581], aging in both sexes (decline in the total androgen pool) [446,447], the use of anti-androgen medications (e.g. for prostatic hypertrophy or cancer) [582], CAIS (women with dysfunctional androgen receptors) [583,584], and autoimmune disease (e.g. Sjögren syndrome, systemic lupus erythematosus, rheumatoid arthritis) [391,430,585].

As an example, researchers have discovered that patients taking anti-androgen therapy, compared with controls, have significant alterations in their meibomian glands, including orifice metaplasia (i.e. a condition defined as an abnormal growth and keratinization of duct epithelium) [586], a decreased quality of secretions, a marked change in the neutral lipid profile of meibum, and a morphological appearance consistent with severe disease [540,541]. Many of the lipid alterations are "all" or "none," the same in both eyes and feature characteristic shifts in fatty acid patterns [541]. Moreover, patients have a significantly greater frequency of tear film (i.e. debris, abnormal menisci, instability), conjunctival (i.e. tarsal injection, inferior staining), corneal (staining), and lid (i.e. irregular posterior lid margins, sleeves, collarettes) abnormalities, as well as increased ocular surface symptoms (i.e. light sensitivity, painful eyes, blurred vision) [540]. These observations extend those of another report, which linked leuprolide acetate treatment (i.e. to reduce testosterone levels) with ophthalmic problems and blurred vision in some patients [587]. In addition, these results may explain why a significant association exists between the use of medications to treat benign prostatic hyperplasia and DED [35].

Investigators have also discovered that androgen receptor dysfunction in CAIS individuals is associated with a significant increase in DED signs and symptoms [542]. These include a significantly reduced tear meniscus, a significantly increased degree of lid erythema, telangiectasia and keratinization, a significantly higher frequency of meibomian gland orifice metaplasia and irregular posterior lid margins, as well as a reduced meibum quality. These CAIS individuals also have striking changes in the neutral and polar lipid patterns of their meibomian gland secretions, compared to those of normal male and female controls [10]. In addition, aging in men and women is associated with a significant decrease in the quality of meibum and a significant increase in the frequency of metaplasia of meibomian gland orifices [112,588]. Aging is also accompanied by marked alterations in the polar and neutral lipid profiles of meibomian gland secretions [112,588]. These observations were made by comparing 37 and 70 year-old people, and this time period between the 4th and 8th decades coincides with a dramatic decline in androgen levels in both sexes [447]. For comparison, sebaceous gland function decreases with age [589]. This age-associated dysfunction has been correlated with both an atrophy of acinar epithelial cells and a reduction in serum androgen levels [589]. In fact, the age-related cellular shrinkage in certain sebaceous glands has been directly correlated with attenuation in androgen levels in the surrounding skin [589].

Additional studies have linked androgen deficiency with MGD and evaporative DED. Researchers have found that non-autoimmune DED patients with MGD are androgen-deficient [590], and others have discovered that the topical application of DHEA to a human, as well as to rabbits and dogs, stimulates the elaboration and release of meibomian gland lipids and prolongs the tear film breakup time [544]. Furthermore, investigators have reported that decreased serum concentrations of testosterone are



more prevalent in women with DED and correlate with the subjective severity of ocular symptoms [526], and that serum testosterone concentrations correlate positively with meibomian gland secretion volume and orifice diameter in pre- and post-menopausal women, respectively [591,592]. This latter correlation is of interest, given that serum testosterone levels represent < 0.2% of the total androgen pool in women [450].

In summary, these findings indicate that the meibomian gland is an androgen target organ, that androgens promote lipogenesis and suppress keratinization within this tissue, and that androgen deficiency may lead to MGD and evaporative DED. This interrelationship between androgen deficiency, meibomian gland dysfunction and evaporative DED may help to explain why topical or systemic androgen administration have been reported to alleviate the DED signs and symptoms in women and men [394,396,397,410,411,461,462,544]. Consistent with these data are Phase 2 clinical trial results, which show that treatment of MGD with topical testosterone improves the quality of meibomian gland secretions and reduces ocular discomfort [593].

### 3.1.3. Androgen regulation of the cornea and conjunctiva

**3.1.3.1. Androgen influence and mechanism of action.** Androgens have been reported to stimulate the proliferation, dynamics and/or immune response of the cornea and conjunctiva [155,594–599]. Conversely, androgen deficiency has been linked to the development of corneal and conjunctival epitheliopathies [369,393].

The mechanism of androgen action in the cornea and conjunctiva appears to involve the local, intracrine synthesis of androgens from adrenal precursors (e.g. DHEA), binding to saturable, high-affinity and androgen-specific receptors, control of gene transcription, and ultimately modulation of translation. Human corneal and conjunctival epithelial cells contain all the steroidogenic enzyme mRNAs necessary for the intracrine synthesis and metabolism of androgens [427,459,600,601]. In addition, androgen receptor mRNA and protein are present in epithelial cell nuclei of the cornea and conjunctiva [389,427,602–605], and DHT is known to modulate the expression of almost 1500 genes in primary human corneal epithelial cells [606], as well as over 3000 genes in immortalized human conjunctival epithelial cells [560].

In primary human corneal epithelial cells DHT upregulates genes encoding syntenin (e.g. regulates transmembrane-receptor trafficking, tumor-cell metastasis, neuronal-synapse function and NF-kappaB activation pathways), decorin (e.g. inhibits angiogenesis) and vimentin (e.g. helps maintain cell integrity) and suppresses genes producing tubulins (e.g. promote angiogenesis) and matrix metalloproteinase 9 (e.g. degrades collagens) [606]. DHT treatment also exerts a significant impact on many ontologies, including oxidative phosphorylation, transmembrane ion transport and cellular localization [606]. In contrast, DHT treatment of immortalized human conjunctival epithelial cells enhances the expression of genes involved in epithelial development, regeneration, wound healing and cell migration (e.g. matrix metalloproteinase), and suppresses those related to the immune response (e.g. chemokine (C-X-C motif) ligand 6) and mitotic cell cycle (e.g. septin 4, endothelin 1) [560].

**3.1.3.2. Clinical relevance of androgen influence on the cornea and conjunctiva.** Androgen treatment has been demonstrated to stimulate mitosis, repair defects and facilitate wound healing, and to suppress angiogenesis and dystrophies in the cornea [155,594–598]. Moreover, androgens have been shown to alter the progression of allergic conjunctivitis [599] and to suppress the LPS-induced secretion of LTBA in both human corneal and conjunctival epithelial cells [575].

In contrast, androgen deficiency is associated with corneal and conjunctival damage. Patients on anti-androgen therapy have a significant decrease in their tear film breakup time (TBUT), as well as a significant increase in the degrees of corneal fluorescein and rose bengal staining and inferior bulbar conjunctival rose bengal staining [540]. In addition, CAIS individuals have increased lid erythema, telangiectasia and keratinization [542], a greater frequency of irregular posterior margins, conjunctival erythema, and bulbar and tarsal injection [542], and a decreased expression of MUC1 [607]. This impact of androgen deficiency on the ocular surface has also been observed in animals. Investigators discovered reduced TBUT, increased corneal fluorescein staining, shorter and flattened corneal epithelial microvilli, and loose intercellular desmosomes in mice after orchietomy [393]. These alterations were largely resolved following DHT treatment [393]. For comparison, complete androgen receptor absence in mice leads to premature ovarian failure, a condition in humans that is linked to heightened ocular surface damage, characterized elevated corneal fluorescein staining, increased conjunctival lissamine green staining, and DED symptoms [519]. Conjunctival, but not corneal, changes were also found in rabbits following orchietomy, including increased rose bengal staining and decreased numbers of goblet cells [369].

Androgens have also been linked to the development of keratoconus in mice [443], and hyperandrogenism to an “itchy-dry eye” in women with polycystic ovary syndrome [608,609]. The polycystic ovary syndrome patients had a reduced TBUT, conjunctival hyperemia and increased goblet cell density [608]. However, the extent to which the ocular findings in polycystic ovary syndrome relate to excess androgen levels is unclear. Polycystic ovary syndrome patients also have insulin resistance, which leads to Type II diabetes, which in turn leads to DED [610–612].

### 3.1.4. Androgen role in sex-related differences of the ocular surface and adnexa

Androgen influence seems to account for many of the sex-related differences in the anatomy, molecular biology, physiology, immunology and disease susceptibility of the lacrimal gland in a variety of species, including mice, rats, hamsters, guinea pigs, rabbits and humans [12,14,82,87,100,102,103,105,195,199,202,205,249,351,379,390,395,400–405,407,409,412,417,613–617]. For example, of the hundreds of genes more highly expressed in lacrimal glands of male, as compared to female, mice, greater than 80% of those genes are also up-regulated by testosterone [351]. Similarly, of the hundreds of genes that are expressed significantly less in glands of male, relative to female, mice, more than 65% of those genes were also down-regulated by testosterone. Further, the top 10 to 14 biological process, molecular function and cellular component ontologies regulated by testosterone in mouse lacrimal glands are identical to those found to be influenced by sex [83,351]. In contrast, estrogen (15%) and progesterone (2%) influence only a small percentage of those lacrimal gland genes [352] reported to vary significantly between male and female mice [83].

Androgens are also known to exert sex-specific actions in the lacrimal gland [90]. Researchers have reported that sex steroid actions on the transcription of certain genes, the translation of the corresponding proteins, and the development of paradoxical inflammation in the lacrimal gland are sex-biased [11,64,86,90,105,480,618]. Such sex specificity of hormone action is not unusual. Sex steroids induce sex-specific effects in many cells (e.g. neutrophils, antigen-presenting cells and preadipocytes) and tissues (e.g. muscle, liver, hippocampus, spinal cord and vasculature) [618–625]. Sex steroids may also elicit opposite [626–630], and even antagonistic [631], influences, such as on sebaceous gland activity. Androgens and estrogens may often induce opposite responses in the lacrimal gland [90]. These responses may contribute

to estrogen's possible pro-inflammatory [376], and androgen's anti-inflammatory [358,374,376,384], effects in lacrimal tissue in Sjögren syndrome.

The differential action of sex steroids, as in other sebaceous glands [548,552,632–634], may also play a role in the sexual dimorphism in the morphological appearance, gene expression, neutral and polar lipid profiles, and secretory output of the meibomian gland [6–8,18,19,35,112,113,188,543,588,635]. Androgens mediate almost 30% of the sex-associated differences in gene expression of the mouse meibomian gland [8]. In addition, androgens may contribute to the significant sex-related differences found in the lipid profiles of human meibomian gland secretions [112]. Analogous sex-associated differences exist in the mass/charge ratios of some neutral, but especially polar lipids in human meibum, and these have been linked to the presence of functional androgen receptors [543].

As in the lacrimal gland, androgens also have sex-specific effects in the meibomian gland. For example, several genes are up- or down-regulated by testosterone in the female, but not male, mouse meibomian gland [90]. In addition, a number of the androgen-regulated genes in female glands are altered in the opposite direction by 17 $\beta$ -estradiol and/or progesterone [90]. These genes could be involved in cell maturation, migration and holocrine secretion in the meibomian gland. If so, this would be consistent with a pro-sebaceous action of androgens and an anti-sebaceous effect of estrogens.

In the human corneal epithelium, males, as compared to females, have a significantly higher expression of genes associated with DNA replication and cell migration [9]. These sex-associated differences may be due to the influence of androgens, given that these hormones are known to stimulate mitosis in the corneal epithelium [155].

### 3.2. Estrogen and progesterone regulation of the ocular surface and adnexa

In contrast to androgen, the role of estrogen at the ocular surface is less well defined, with effects that appear to be tissue-, sex-, and dose-specific.

#### 3.2.1. Estrogen and progesterone presence at the ocular surface

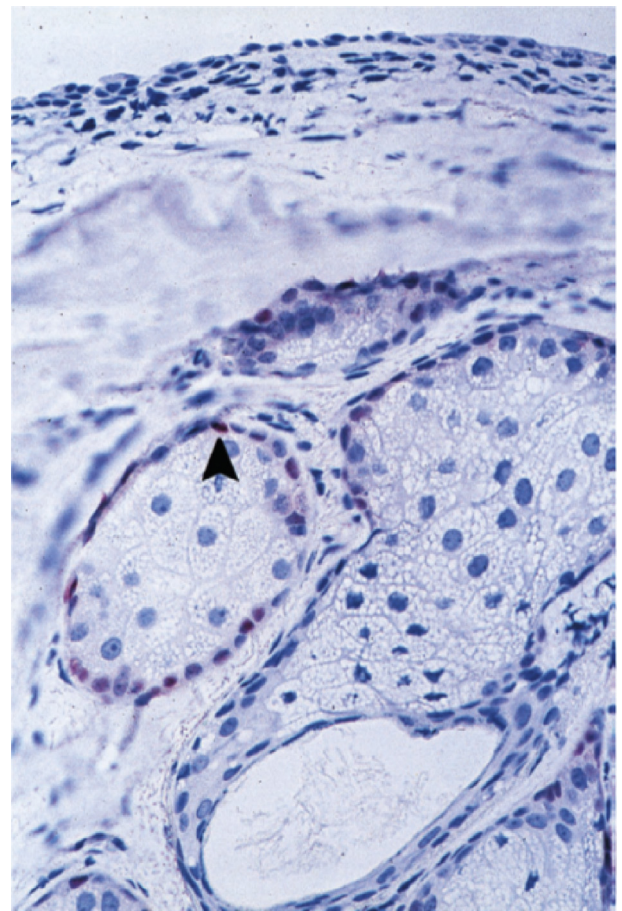
Analysis of intra-tissue estrogen or progesterone levels in ocular tissue has not as yet been undertaken. Both estrogen and progesterone have been detected in human tears, and are reported to be correlated with levels in serum of premenopausal females [636]. A study of progesterone and DHEA found tear levels to be substantially lower than those in serum, with no difference between males and females [637]. Other than these two conference abstracts, no investigation of sex hormones in tears has been published, reflecting the difficulties in detecting low concentration compounds, such as sex hormones, in small volumes of tears.

As is the case for testosterone, there is substantial evidence for biosynthesis and metabolism of estrogen at the ocular surface, which implies that it exerts a biological influence here. Aromatase, steroid sulfatase and hydroxysteroid dehydrogenases (17 $\beta$  HSD-1 and 3; 3 $\alpha$  and  $\beta$  HSD) mRNAs have been identified in human meibomian and lacrimal gland tissue and in immortalized corneal and conjunctival epithelial cell lines [459]. If these mRNAs are translated, this means that human ocular surface tissues possess the enzymes necessary for the conversion of the precursor DHEA-S into estrogen in the process of intracrinology (Fig. 1). Intracellular synthesis and metabolism of sex hormones is a process unique to primates, and as such suggests caution for extrapolation of rodent studies with estrogens to humans.

Intracrine sources account for all estrogen synthesis in

postmenopausal women, the majority of estrogen in men and up to 75% of estrogen synthesis in women prior to menopause [449,638]. In women after menopause, intracrine metabolism is an evolutionary measure which limits exposure of other tissues (specifically the endometrium) to estrogen, while providing estrogen to tissues where it continues to have an important function throughout life, including bone, brain, vascular tissue and presumably the ocular surface. Hence, in postmenopausal women and in men, local rather than systemic estrogen levels would be expected to direct estrogen action in peripheral tissue, including the ocular surface [639]. Circulating estrogen levels in postmenopausal women are the sum of small amounts of 'leakage' from various peripheral tissue sources, and as such, are not a good measure of estrogen influence at the ocular surface. Serum estrogen levels may be more relevant to the ocular surface of women prior to menopause.

Intracrine synthesis of estrogen is dependent on circulating levels of precursors, specifically DHEA, but also testosterone. Levels of serum testosterone are an order of magnitude greater than circulating estrogen levels in postmenopausal women and thus probably an important source of estrogen in peripheral tissues [639]. Thus, further to its direct androgen action discussed in the section above, testosterone has an important influence on estrogen action through its aromatization to estrogen in target tissues, including the ocular surface. In men, serum levels of testosterone are another order of magnitude higher than those in postmenopausal women [639], and thus may be an even more



**Fig. 2.** Cross-section through normal human meibomian glands in the upper eyelid. Immunohistochemical staining shows nuclear positivity for estrogen receptors in basal and parabasal cells of acini (arrow).642 Reprinted from Ophthalmology 107, Esmaili B, Harvey JT, Hewlett B, Immunohistochemical Evidence for Estrogen Receptors in Meibomian Glands, Pages No. 180–184, Copyright 2000, with permission from Elsevier.

important source of estrogen at the ocular surface. Progesterone, in contrast, is primarily formed from cholesterol within the adrenal glands and the ovaries, and its intracrine synthesis is not well described.

### 3.2.2. Estrogen and progesterone receptors at the ocular surface

The presence of estrogen and progesterone receptor mRNA and receptor proteins is further evidence that ocular surface tissues are target sites for these hormones. In humans, mRNA for estrogen receptors (ER) has been identified in meibomian glands [426], lacrimal gland [426,640], cornea [426], bulbar conjunctiva [426,640,641], and in the tarsal plate [640]. It has been confirmed that these mRNAs are translated into proteins. ERs are present in the human meibomian gland, with an age-related increase in number of cells expressing ERs and no sex-related differences in distribution [642,643] (Fig. 2). A similar increase with age has been reported for estrogen and progesterone receptors (PR) in lacrimal gland cells, where they may be more frequent in women [644] (Fig. 3). ER and PR have also been identified in corneal epithelial, stromal and endothelial cells [604] and in bulbar conjunctival epithelium [641]. Conjunctival cells expressing ER or PR have not yet been investigated in men or in postmenopausal women. In human meibomian gland and corneal cells, the majority of ER appear to be the ER $\alpha$  type [604,643], whereas both ER $\alpha$  and ER $\beta$  are expressed in conjunctival epithelium [641]. In other tissues, ER $\alpha$  and ER $\beta$  mediate different functions of estrogens and thus a more complete characterization of the relative distribution of these receptor types at the ocular surface may provide important clues to understanding the role of estrogens in dry eye. Membrane receptors for sex hormones have been identified in human corneal and meibomian gland epithelial cells, and these may also play a role [645].

### 3.2.3. Estrogen and progesterone mechanism of action at the ocular surface

**3.2.3.1. Classic receptor action – regulation of gene expression.** Estrogen and progesterone appear to act directly on their respective receptors to modulate the expression of genes that alter biological processes, molecular functions and cellular components in the lacrimal gland, the meibomian gland and in corneal epithelium in mice [117,352,646]. However, studies to determine whether estrogen and progesterone act through such classical receptors in human ocular surface tissue are yet to be carried out.

**3.2.3.2. Non-genomic action.** Estrogen and progesterone may also act on ocular surface tissue in an indirect fashion, through non-

nuclear signaling pathways or via control of other pituitary hormones [647]. Non-nuclear signaling systems are important when immediate responses are required to maintain homeostasis of the tear film and ocular surface. Whereas the classic genomic mechanisms take hours or days to effect action, non-genomic signaling pathways at the membrane or in the cytoplasm can result in responses that occur much more rapidly, on the order of seconds or minutes [648–650]. Membrane bound estrogen receptors have been identified in human cells in non-ocular tissues [648,649], and receptors distinct from ER $\alpha$ , ER $\beta$  and PR or their isoforms may also be involved [650–653]. The involvement of alternate signaling pathways by estrogen in ocular tissue is supported by a study which showed that estradiol downregulates cyclic AMP signaling in human meibomian gland epithelial cells [654].

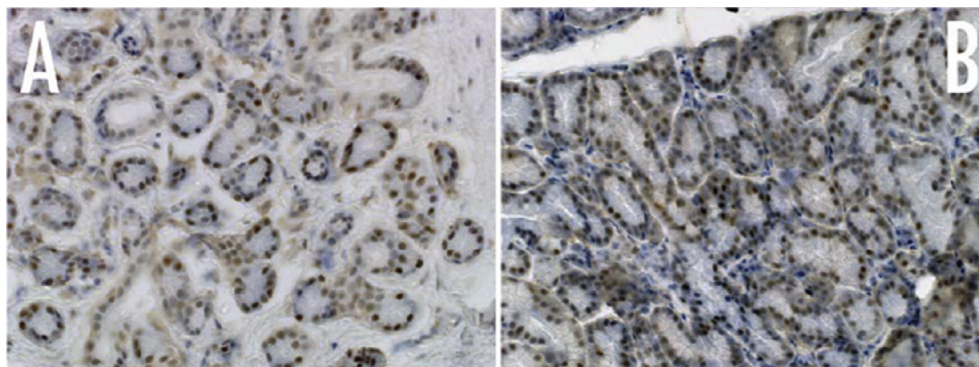
**3.2.3.3. Immunity and inflammation.** Estrogen has well-known effects on the immune system. These actions are often dose- and tissue-dependent and may be completely different depending upon on the estrogen concentration. These differential effects may underlie some of the contradictory results found in studies evaluating estrogen for the treatment of dry eye disease [655,656].

Many types of immune cells, including T and B lymphocytes, dendritic cells, macrophages, neutrophils and natural killer cells express receptors for estrogen, progesterone and testosterone and hence can respond to these hormones [225,226,657]. In general estrogen enhances immune responses promoting production of B cells and antibody, sub-sets of T cells, dendritic cells, M2 macrophages and regulatory cytokines, while progesterone and androgens tend to decrease immunity [34,658]. Estrogens are the most widely studied of the sex hormones in terms of effects on immunity [225,237,245].

For example, estrogens inhibit the differentiation of Th17 cells in mice likely by down regulation of transcription factors including ROR $\gamma$ T [659,660]. This is an interesting observation given the role of Th17 cells in the pathogenesis of dry eye [661] and greater prevalence of the condition in post-menopausal women. Notably estrogen, acting via ER $\alpha$ , inhibits Th1 and Th17 differentiation and is protective in a model of experimental autoimmune encephalomyelitis [662].

### 3.2.4. Estrogen and progesterone influence on lacrimal gland structure and function

Estrogen and progesterone affect the anatomy and physiology of the lacrimal gland, although there is conflicting evidence regarding the nature and extent of this influence. A gene array study of



**Fig. 3.** Estrogen (A) and progesterone (B) receptors in lacrimal gland acinar cells. Variable intensity of expression [644]. Reprinted from Archives Biological Science 63, Gligorijević J, Krstić M, Babić G, Immunohistochemical detection of estrogen and progesterone receptors in the human lacrimal gland, Pages No. 319–324, Open-Access article distributed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

lacrimal glands in mice identified hundreds of genes that are regulated by 17 $\beta$ estradiol and progesterone [352]. The effects of 17 $\beta$ estradiol, progesterone, and 17 $\beta$ estradiol plus progesterone on gene expression were found to be mostly unique to each specific treatment. A study of aromatase knockout mice showed that the absence of estrogen also impacts the expression of numerous genes in the lacrimal gland [89].

Genes influenced by estrogen and/or progesterone treatment in mice included genes involved in transcriptional regulation, cell growth, division and/or maintenance, cell communication, signal transduction, enzyme catalysis, nucleic acid, protein and lipid metabolism, neurogenesis and a diverse array of immune-related genes [352]. Genes stimulated by 17 $\beta$ estradiol included actions in signaling pathways, ion transport, enzyme activities, and membrane aspects, whereas genes suppressed by estrogen were involved in cell organization and biogenesis, cytokine activity, receptor binding, mitochondria, and intracellular components [352]. Administration of progesterone increased expression of genes linked to signal transduction and cell communication and attenuated expression of genes linked to cell growth, maintenance, metabolism, and ion binding [352]. Interestingly, combined 17 $\beta$ estradiol and progesterone treatment increased expression of cell death genes and decreased that of genes related to receptor binding, signal transduction, protein transport, cytokine activity, and development [352]. 17 $\beta$ estradiol and progesterone were found to only modulate a small proportion of genes regulated by androgens, however, these effects were often in a direction opposite to that of androgen [663]. Although caution is urged in applying findings from mice to humans [664,665], these results are supportive of an important role of estrogen and progesterone in the lacrimal gland, which is beyond that of modulation of inflammation. In addition, these gene data represent mRNA levels, and translation into proteins is yet to be determined. However, if translation does occur, and if even some of the findings in mice are transferrable to humans, such alterations in gene activity influenced by estrogen and/or progesterone may be associated with ocular surface effects which lead to dry eye.

It has been proposed that estrogen and progesterone may have an anti-inflammatory influence in the lacrimal gland, and estrogen deficiency from ovariectomy or anti-androgen treatment in animals has been reported to negatively impact the lacrimal gland. In rabbit and mouse models of Sjögren syndrome, absence of estrogenic influence is reported to lead to regressive, inflammatory changes in lacrimal gland tissue, while estrogen administration prevents and/or reverses these changes and promotes lacrimal secretion [513,666,667]. The finding that estrogen with or without progesterone treatment influences the expression pro-inflammatory genes in the mouse lacrimal gland may support this thesis [352]. However, a more recent study of aromatase knockout (ArKO) mice, demonstrated that although estrogen absence exerted a substantial impact on expression of thousands of genes in the lacrimal gland, it did not significantly induce upregulation of genes related to inflammatory pathways [89]. In the same study, no histological evidence of lacrimal gland inflammation was observed, and tear volume was not reduced in ArKO mice. This lack of effect on tear volume is different than found following aromatase inhibitor treatment of postmenopausal women in which development of dry eye was reported [668–670]. It may be that increased circulating levels of androgens and other hormones in the sera of ArKO mice contribute to the difference found with the human studies [89].

In contrast, a number of studies in humans and animals show that estrogen and/or progesterone have a negative influence on the lacrimal gland. Estrogen action on the lacrimal gland promotes inflammation and autoimmune disease in some conditions [376].

Estrogen treatment of rabbits and mice increased the activity, level and expression of MMP-2 and -9 in the lacrimal gland [464,671]. This effect was not apparent in mice treated topically [464]. Other studies found no effect of estrogen treatment on lacrimal gland structure or protein secretion in humans or rats [12,14,412,672,673].

The findings from numerous investigations, in rodents and in humans, of the influence of estrogen on the structure and function of the lacrimal gland are summarized in Table 6 [19,60,89,350,352,355,464,513,519,647,666,668–671,674–683]. Contradictions apparent in these findings may be explained to some degree by differences in study design, treatment dosage, patient cohort or animal models (including sex-difference, Section 2.4). Conflicting results may also be due to the influence of other hormones (see Sections 3.1., 3.3–3.6) and as yet unidentified pathways that influence lacrimal gland function.

### 3.2.5. Estrogen and progesterone influence on meibomian gland structure and function

The nature of estrogen's and progesterone's influence on meibomian gland function is yet to be completely understood. Gene array studies in mice suggest that estrogen and progesterone have multiple effects on meibomian gland function and may possibly decrease lipid synthesis. The evidence in humans from population and clinical studies may support this contention (see Section 3.2.7). Estrogen and progesterone have also been reported to alter the morphology of mouse meibomian glands, involving changes in the area and/or number of acinar epithelial cells [6].

The meibomian gland is a large sebaceous gland, and there is substantial evidence supporting the negative impact of estrogen on the structure and function of sebaceous glands in other tissues and in various species [20,213,631,632,684–686]. The use of estrogen or combined estrogen and progesterone treatment to reduce sebum production in acne vulgaris is a well-known example [687,688]. Of note, the impact of estrogen and progesterone in sebaceous glands is tissue-, sex- and species-specific [213].

In the meibomian gland, estrogen and progesterone modulate expression of genes that influence biological processes, molecular function and cellular components in mice [117,646]. Importantly, estrogen opposes some actions of androgen in the meibomian gland. Estrogen suppresses genes associated with support of lipid production and upregulates genes which have the opposite effects [646,663]. In mice treated with 17 $\beta$ estradiol, progesterone or a combination, a gene microarray study showed that both hormones modified expression of almost 200 genes in the meibomian gland [646]. Overall, estrogen might have a negative influence on meibomian gland lipid generation and secretion due to its downregulation of genes involved in lipid biosynthesis, acinar cell maturation, migration and holocrine secretion, and its upregulation of genes involved in lipid and fatty acid metabolism [646]. In addition, estrogen was shown to modulate, albeit in smaller numbers, genes associated with promotion of lipid production, immune factors, tyrosine kinases and steroidogenesis [646]. Whereas 17 $\beta$ estradiol upregulated and downregulated equal numbers of genes, the impact of progesterone treatment in the mouse meibomian gland was of smaller magnitude and mostly via downregulation of gene expression [646]. Progesterone's most prominent influence was in suppression of genes regulating protein, macromolecule biosynthesis and ribosome biogenesis. Combined 17 $\beta$ estradiol/progesterone treatment mostly mirrored the effects of either hormone administered alone, but also influenced additional gene ontologies, and in some cases had opposing effects on gene expression [646]. Overall, these various steroid treatments led to many analogous, opposite, or unique effects on gene expression [646].

A subsequent gene microarray study of wild type and ArKO mice showed that aromatase (and by extension estrogen) presence and absence significantly influenced the expression of over 1000 genes in the meibomian gland, and that the nature of these effects was highly sex-dependent [117]. Of note, aromatase deficiency in female mice resulted in significantly increased expression of genes encoding a variety of immune functions, whereas mitotic ontologies were increased in male mice [117]. The sex-related variations were similar in ArKO and WT mice, indicating that aromatase and estrogen do not play a primary role in the sex-related differences in the meibomian gland [117]. Interestingly, the majority of genes significantly influenced by estrogen absence in the meibomian gland were different to those identified in the lacrimal glands [89], which is further evidence for the tissue-specific nature of the impact of estrogen on the ocular surface.

Aromatase (and thus estrogen) deficiency did not have an effect on the histology of meibomian glands in male of female mice, and no signs of inflammation were evident [117]. In contrast, an earlier study reported changes in meibomian gland morphology, involving changes in the area and/or number of acinar epithelial cells, in mice treated with estrogen [6]. The difference may relate to the increased serum levels of androgens and other hormones in the

ArKO mice [89].

As in the lacrimal gland, results in mice may not be directly transferable to humans [664,665], and the findings discussed require confirmation in human trials. Evidence of estrogen suppression of cAMP signaling in, and the proliferation of, immortalized human meibomian gland epithelial cells supports the gene array findings in mice [654]. Furthermore, a number of clinical studies report relationships between higher estrogen levels and reduced tear stability and meibomian gland secretion [313,355,689]. In contrast however, improved tear stability has been reported in studies of women undergoing hormone replacement therapy (HRT) and topical estrogen administration (Table 7) and these are discussed below (see Section 3.2.7).

### 3.2.6. Estrogen influence on corneal and conjunctival structure and function

In addition to the lacrimal and meibomian glands, it is likely that estrogen has a direct influence on the epithelial cells of the cornea and conjunctiva. Although the weight of the evidence is on estrogen's impact on conjunctival tissue, the inherent difficulty of obtaining corneal cells from living humans has limited the *in vivo* studies examining the effect of estrogen on the human cornea.

**Table 6**  
Reported influence of estrogen treatment, ovariectomy, estrogen absence or anti-estrogen administration on the structure and function of the lacrimal gland in mice, rats, hamsters, rabbits and humans.

	Estrogen + (e.g. estrogen treatment, HRT)	Estrogen – (e.g. ovariectomy, estrogen absence, anti-estrogen administration)
Lacrimal gland regression	✓	✓
Lacrimal gland weight or morphology	no effect, glandular appearance restored to a “female” type	no effect, morphological appearance changed to a “male” type
Alteration in structural profile to a “neutral” type		✓
Lacrimal gland histology		no effect
Glandular tissue		↓
Acinar cell disruption & vacuolization, necrosis	↓	↑, no effect
Connective tissue		↑
Lymphocyte infiltration		↑, no effect
DNA and RNA levels	↑	
DNA degradation	↓	↑, no effect
Gene expression (numerous genes)	altered	altered
Leucine aminopeptidase amount	↑	
PAS-staining	↓	
Total activity of β-adrenergic receptors	↑	↓
Total activities of cholinergic receptors & Na <sup>+</sup> , K <sup>+</sup> -ATPase	↓	↓
Acid & alkaline phosphatase activities	↓	↓
Lacrimal fluid peroxidase	↑*, ↓*, no effect	
Total protein content	no effect	altered
Membrane-bound protein content		altered
20 kDa protein content	↓	altered
Specific protein secretion	no effect	
Phenylephrine (α-adrenergic agonist)-induced peroxidase & total protein secretion		↑
Antagonism of certain androgen effects	✓*	
Inflammation	↑ ↓	no effect, ↑(SS)*
Tear output	↑*	no effect, ↑(male only)
	↓*, no effect*	
Dry eye improvement	✓	
Dry eye development	✓*	✓*

Check marks indicate that an effect of estrogen supplementation or absence was identified. Upward (↑) and downward (↓) arrows indicate direction of effect. Red text indicates negative impact on DED. Multiple entries signify conflicting results from different studies. Findings in humans are marked with an asterisk (\*). SS, Sjögren Syndrome. Table adapted from Sullivan et al. [647] and updated [19,60,89,350,352,355,464,513,519,666,668–671,674–683].

**Table 7**  
Effects of estrogen and progesterone supplementation on the ocular surface of postmenopausal women.

Reference	Treatment (number of participants)	Duration	Administration	Control	Symptoms	TBUT	Schirmer test	Other parameters
<b>Positive effect on dry eye</b>								
Akramian et al. [676]	E (n = 10)	4w	topical ointment	placebo	no effect	↑	↑	
Sator et al. [675]	E + P (n = 42)	4 m	E topical drops + oral E + P	artificial tears* + oral E + P	↓(topical E group)		↑(topical E group)	
Vavilis et al. [696]	E (n = 7) E + P (n = 4)	4 m	transdermal transdermal + oral	no control				improvement in conjunctival epithelium maturation changes
Affinito et al. [679]	E + P (n = 25)	3, 6 m	transdermal	untreated	↓		↑	
Guaschino et al. [678]	E + P (n = 40)	12 m	oral	untreated			↑	
Pelit et al. [697]	E + P (n = 17)	3 m	oral	no control		↑	no effect	↑ goblet cell density
Altintas et al. [677]	E + P (n = 15)	2 m	oral	untreated		↑	↑	
Moon et al. [680]	E + P (n = 36)	1, 3 m	oral	no control	↓		↑	
Coksuer et al. [681]	E + P (n = 34)	6 m	oral	no control	↓	↑	↑	
Okon et al. [682]	HRT (n = 60)	3, 6, 12 m	oral	no control		↑	↑ (6, 12 m)	
<b>Negative effect on dry eye</b>								
Golebiowski et al. [689]	E (n = 10)	2 m	transdermal	placebo	↑	no effect	no effect	no effect on tear osmolarity, MG function, lid morphology, corneal or conjunctival sensitivity
Uncu et al. [674]	E (n = 5) E + P (n = 19)	6, 12 m	Transdermal oral	no control			↓ (12 m) worse E group	
Erdem et al. [712]	E + P (n = 20)	3 m	oral	tear supplement	↑	no effect	no effect	possible effect on MG
Shaharuddin et al. [703]	E (n = 11) E + P (n = 19)	single time point	not stated	untreated				↑ frequency of dry eye signs
Schaumberg et al. [60]	HRT (n = 15681)	population study	various	untreated				↑ dry eye prevalence with HRT, worse with E only
Chia et al. [19]	HRT (n = 655)	population study	various	untreated				↑ dry eye prevalence with HRT
<b>Equivocal effect on dry eye</b>								
Evans et al. [704]	E (n = 10)	single time point	transdermal or implant	untreated	↓			no difference in osmolarity
Kuscu et al. [707]	E + P (n = 10)	6 m	oral	no control	no effect	no effect	no effect	improved MG assessment, ↑ tear IgA, no effect on tear lysozyme
Taner et al. [713]	E + P (n = 25)	6 m	oral	untreated		no effect	no effect	no effect on conjunctival cytology
Piwkumsribonruang et al. [708]	E + P (n = 21)	3 m	transdermal + oral	placebo	no effect	no effect	no effect	
Jensen et al. [705]	HRT (n = 25)	single time point	various	untreated	↓		no effect	
Lekskul et al. [714]	HRT (n = 38)	single time point	oral	untreated		no effect	no effect	

Key: E, estrogen; P, progesterone; HRT, hormone replacement therapy (various constituents); TBUT, tear break up time; MG, meibomian gland; m, months; w, weeks. \*benzalkonium chloride preserved. Table adapted from Truong et al. [350] and updated.

**3.2.6.1. Cornea.** In human corneal epithelial cell culture, estrogen treatment of primary cells results in reduction of IL-6 and IL-8 mRNA levels and has no effect on IL-1 $\beta$ , matrix metalloproteinase (MMP) -2 or MMP-9 gene expression [690]. In contrast, in immortalized (SV40) cells, estrogen treatment has been shown to upregulate expression of a variety of proinflammatory cytokine genes (IL-1 $\beta$ , IL-6, IL-8, GM-CSF) and MMP-2, -7 and -9 genes [690,691]. The increased expression of MMP-2 and MMP-9 mRNA was not accompanied by an increase in protein translation and consequent secretion. Interestingly in another study, when SV40-immortalised corneal epithelial cells were exposed to a hyperosmolar medium (450 mOsm/L) simulating the conditions of dry eye, pre-treatment of cells with estrogen inhibited the mRNA expression and production of the proinflammatory cytokines IL-6, IL-1, TNF- $\alpha$  [692]. Another study of SV40-immortalised human corneal epithelial cells showed faster rates of cell migration in response to estrogen, as well as higher (dose-dependent) levels of epidermal growth factor and increased gene expression of fibronectin [693]. This suggests estrogen could have a beneficial effect

on wound healing, although these findings are yet to be replicated in primary cells. The SV40-immortalised cell line does not represent normal corneal cells with respect to the estrogen regulation of cytokine and MMP responses, perhaps as a result of differences in ER $\alpha$  and ER $\beta$  expression [690].

Changes in corneal sensitivity have been reported in relation to altered estrogen levels during the menstrual cycle, albeit these are also conflicting. Reduced corneal sensitivity has been reported to occur at the estrogen peak in the ovulatory phase [220] and also during the menstrual phase during which lowest estrogen levels are reported [694]. No alterations in corneal sensitivity were detected following transdermal estrogen treatment in a group of post-menopausal women with dry eye [689].

**3.2.6.2. Conjunctiva.** Utilizing conjunctival smears processed with the Papanicolaou technique ("Pap smear"), epithelial cell morphology was examined in menstruating and postmenopausal women [695] and also following HRT [696]. Cyclical changes were demonstrated in the conjunctival cells which were similar to (albeit

milder than) those seen in the cells of vaginal epithelium, with a shift towards more mature (i.e. intermediate/superficial) cells during the estrogen peak at ovulation. During the menstruation phase with lowest circulating estrogen levels, parabasal cells dominated, as they did in the conjunctival smears of the postmenopausal group, which also reflected the pattern seen in vaginal cells. A subsequent study demonstrated an increased proportion of mature cells in postmenopausal women following transdermal estrogen and progesterone treatment [696]. Similar cyclical variations with menstrual cycle were reported in a later study [313]. Increased conjunctival goblet cell density has also been reported following HRT [697]. A recent report did not find sensitivity changes following transdermal estrogen treatment in a group of postmenopausal women with dry eye [689].

### 3.2.7. Clinical relevance of estrogen and progesterone action in DED

The clinical evidence regarding the influence of estrogen and progesterone on dry eye is inconsistent, with epidemiological evidence suggesting a negative impact with exogenous hormone administration in women, whereas smaller intervention studies show a range of effects following treatment, which may be related to dose and menopause status or ovarian function. Observational studies suggest circulating estrogen levels may have differing effects in pre and postmenopausal women. Apart from epidemiological investigations, studies of estrogen effects on dry eye have been carried out exclusively in women.

Although a common assumption exists in the literature that menopause is associated with increased occurrence of dry eye, a closer inspection shows that definitive evidence is lacking. As discussed above (Section 2.2), the prevalence of dry eye is higher in females than males across the lifecycle and dry eye prevalence increases gradually with age in both men and women [18,35]. Such a gradual increase in both sexes is perhaps more in keeping with the gradual decrease in serum androgen levels which occurs with age rather than the abrupt decline in ovarian estrogen at menopause. However, more dry eye symptoms were demonstrated in a study of 17–43 year old women with premature ovarian failure, than in an age-matched control group [519], alluding to a positive role for ovarian estrogen. Three reports of increased risk of dry eye in postmenopausal women undergoing aromatase inhibitor treatment for breast cancer highlight the importance of peripheral estrogen synthesis in ocular surface homeostasis [668–670].

Two observational studies of postmenopausal women have shown higher endogenous serum estrogen levels to be associated with increased osmolarity, reduced tear secretion (Schirmer I) and stability, and with meibomian gland dysfunction [355,689]. In accord with these findings is reports of reduced tear stability (measured using Schirmer I) [466] and higher maturation of conjunctival epithelial cells during the ovulation phase of the menstrual cycle, when serum estrogen levels peak [313]. However, reduced tear stability was not confirmed in another study [312], and neither study showed changes in tear secretion (Schirmer I). Both studies reported higher symptoms of dry eye during the estrogen peak at ovulation and during the late luteal phase when levels of progesterone are also high. Alterations in corneal sensitivity during the menstrual cycle have also been reported [220,694]. Other investigations have not found variation of ocular discomfort or of tear function measures including tear secretion, stability, osmolarity and evaporation, over the course of a menstrual cycle [683,698–701].

A large population-based study of 25,665 postmenopausal women from across the U.S. showed higher risk of dry eye for women using hormone replacement therapy (HRT), particularly with estrogen-only therapy [60]. HRT use was also identified as a risk factor in a smaller Australian study [19]. These epidemiological

findings are supported by smaller clinical studies showing systemic estrogen and progesterone administration during HRT to have a negative effect on symptoms [689,702], tear secretion [674] and dry eye signs [703] (Table 7). Other clinical evidence, however, suggests estrogen supplementation with or without progesterone improves dry eye symptoms [675,679–681,704,705], tear secretion [675–681,706] and tear stability [676,677,681,697,706], and may have positive effects on the conjunctiva [696,697] and possibly on the meibomian glands [707] (Table 7). Importantly, however, none of the studies reporting improvement in symptoms were placebo controlled. Of the three placebo-controlled studies, one showed a worsening of symptoms with treatment [689] and two reported no change [676,708]. Also important to note is that studies of topical application of estrogen to the ocular surface or ocular adnexa showed improvement in tear function, although the estrogen doses used in both studies were 2500 and 300,000 times the normal physiologic levels of estrogen [675,676,709]. As an additional consideration, all patients in the Sator study were treated with systemic estrogen and progesterone [675]. The topical placebo, but not topical estrogen, group was given benzalkonium chloride-containing drops. It is not clear to what extent the results reflect a toxic effect of benzalkonium chloride, as compared to a positive effect of estrogen.

Estrogen supplementation in women pre-menopause is also not conclusive. Anecdotal evidence and case reports imply that oral contraceptive use may be associated with increased symptoms of dry eye and contact lens intolerance. However these observations have not been confirmed by formal investigations, which found that oral contraceptive use did not impact ocular symptoms, tear osmolarity, tear secretion, tear stability or other aspects of tear physiology [698,699,710]. Of possible relevance is a case series of two instances of dry eye and reduced tear secretion in young women, associated with development of Sjögren syndrome following high dose estrogen treatment [711].

Some of these contradictions may be explained by estrogen inducing differential actions over a range of concentrations and on different tissues of the ocular surface. Estrogen has been shown to have differential effects on inflammation in various tissues at differing circulating levels (see Straub [656] and Lang [655] for review), although this has yet to be shown in the eye. The effects of estrogen on the ocular surface need to be considered in context of the concurrent actions of other hormones, including progesterone and in particular the androgens. Combination estrogen/progesterone treatment has differential effects on gene expression in lacrimal and meibomian glands compared to estrogen alone (discussed above). Thus the relative ratios of the levels of these hormones should also be taken into account in clinical assessments. It may be the reduction in androgen action, rather than increased estrogen action *per se*, that is responsible for the higher prevalence of dry eye in women. Most importantly, due to the intracrine synthesis of estrogen particularly after menopause, assessment of local tissue levels of estrogen, rather than serum levels, is more appropriate and may yield more consistent clinical relationships with indicators of dry eye.

A more consistent approach to study design will allow more definite conclusions to be drawn. Of the treatment studies summarized in Table 7, only two are placebo controlled, an essential component when assessing a condition in which subjectively reported symptoms are a primary feature. Other clinical studies are underpowered to examine dry eye indices, including symptoms and tear function, which have large variability. The use of convenience samples of women undergoing HRT has made it difficult to control for treatment dose and duration or patient cohort. Importantly, few studies report measures of treatment compliance or bioavailability such as circulating or local hormone levels.

### 3.3. Glucocorticoid regulation of the ocular surface and adnexa

#### 3.3.1. Glucocorticoids in ocular surface physiology and inflammation

Glucocorticoids (GC) are important endogenous regulators of the inflammatory response exerting potent anti-inflammatory effects in the body through the activation of the glucocorticoid receptor (GR), a nuclear hormone receptor that modulates gene transcription. The GR is a cytoplasmic protein, residing in an “aporeceptor” complex together with heat shock proteins 70 and 90, immunophilins, and p23 [715]. When the GR is associated with the GC ligand, the aporeceptor partially dissociates, enabling the GR to translocate to the nucleus, where it binds genomic GC-response elements (GRE) and regulates transcription of associated genes. Depending on the type of GRE activated, the activity of GC can be either activation or repression of gene transcription. The activity of the GR depends on the presence of cofactors (coactivators or corepressors), essential to conduct the signal to the transduction machinery and to chromatin, to obtain the necessary nucleosome remodeling and altered DNA topology. Among these cofactors, the most studied are the p160 proteins family, including the glucocorticoid receptor-interacting protein 1 responsible for the activation of the so called tethering GRE, which mediate the inhibitory effects of GC on transcription and, therefore, their immunosuppressive/immunomodulating activity [716–721].

As the primary physiological anti-inflammatory and immunosuppressive hormones in mammals, GC can interfere with multiple steps of both the innate and adaptive immune responses [722]. Also synthetic derivatives of these hormones are widely prescribed as anti-inflammatory agents including for ocular surface conditions. Topical GC are used as short-term therapy for the treatment of moderate [723] and severe dry eye disease [724–726] and related ocular surface inflammation. However, long-term use of these hormones may increase the risk for the development of infection, glaucoma and cataracts.

Under physiological conditions, endogenous GC may exert an autocrine effect in cells such as epithelial cells and fibroblasts, contributing to immunoprotection of the ocular surface mucosa through cortisol production [727]. Two different systems control cortisol production in the body: the hypothalamic-pituitary-adrenal axis for systemic production and the 11 $\beta$ -hydroxysteroid dehydrogenases (11 $\beta$ -HSDs) for local production. The latter is the most important system for the regulation of the inflammatory/anti-inflammatory activity taking place in peripheral tissues such as the ocular surface. Two main GC are produced in the cells: cortisone and cortisol, the latter being the active form. The local production of cortisol depends on the activity of enzymes, such as the 11 $\beta$ -HSDs, which regulate the production of cortisol from cortisone, a reaction dependent on NADPH as cofactor [728], and vice versa. Two 11 $\beta$ -HSD isoforms have been described: 11 $\beta$ -HSD1, which promotes the production of cortisol and 11 $\beta$ -HSD2, which converts cortisol to inactive cortisone [729,730]. The balance between the production of cortisone and cortisol is a key point in the control of the events leading to activation of innate and adaptive immune responses and 11 $\beta$ -HSD1 has been shown to play a fundamental role in helping balance monocyte maturation and immune-cell function with the control of tissue damage consequent to the inflammatory process [731,732]. At the ocular surface, 11 $\beta$ -HSD1 has been found localized to the basal cells of the central corneal epithelium while the serum and glucocorticoid regulated kinase 1 (SGK1), a target gene for the GR, was localized into proliferating limbal cells [716]. At the ocular surface, under physiological conditions, the autocrine synthesis of cortisol by the corneal epithelium contributes to immunoprotection. Primary cultures of human corneal epithelial cells, fibroblasts and allogeneic macrophages (M1) have been shown to be

capable of generating cortisol (fibroblasts more so than epithelial cells) [727].

#### 3.3.2. Glucocorticoid effects on the lacrimal gland

The action of GC on ocular surface and adnexal tissues is concentration dependent. In experimental conditions low amounts of GC are essential for the maintenance of rat lacrimal gland acinar epithelial cells *in vitro* [364], while physiological levels of GCs increase acinar cell synthesis of proteins such as the C3 component of prostatic binding protein, a potent immunosuppressive agent, and cystatin-related protein [381,647] as well as enhancing the androgen-induced production of secretory component [364]. Higher concentrations of GC promote C-reactive protein and C3 component of prostatic binding protein synthesis [381,647], but have no effect on basal secretory component output, and suppress the androgen related secretory component response [364,378]. The mechanism of action of GC on the lacrimal gland may depend on the presence of specific receptors since a glucocorticoid receptor mRNA was identified in human lacrimal tissue [733].

#### 3.3.3. Glucocorticoids and sexual dimorphism

Since inflammation is a core component of a variety of sexually dimorphic diseases, such as autoimmunity, it was postulated that GC exert their anti-inflammatory effects in a sexually dimorphic manner and this may be the underlying mechanism by which men and women respond differently to inflammation [734]. The mechanisms through which GC exert their actions in a sexually dimorphic manner are complex. GC and estrogen receptors can interact and modulate downstream signaling, for example estrogens antagonize GC induction of the glucocorticoid-induced leucine zipper (GILZ) gene, an important mediator of GC anti-inflammatory actions. Hence, estrogen antagonism of GC receptor signaling could be responsible for the less efficient response of females to corticosteroid therapy [735]. A further possibility is a differential expression of sex-specific coactivators and transcription factors. For example females and males express different coregulatory molecules: prohibitin 2 and mediator complex subunit 12 are more expressed in females, while TATA box-binding protein is more expressed in males [734–736].

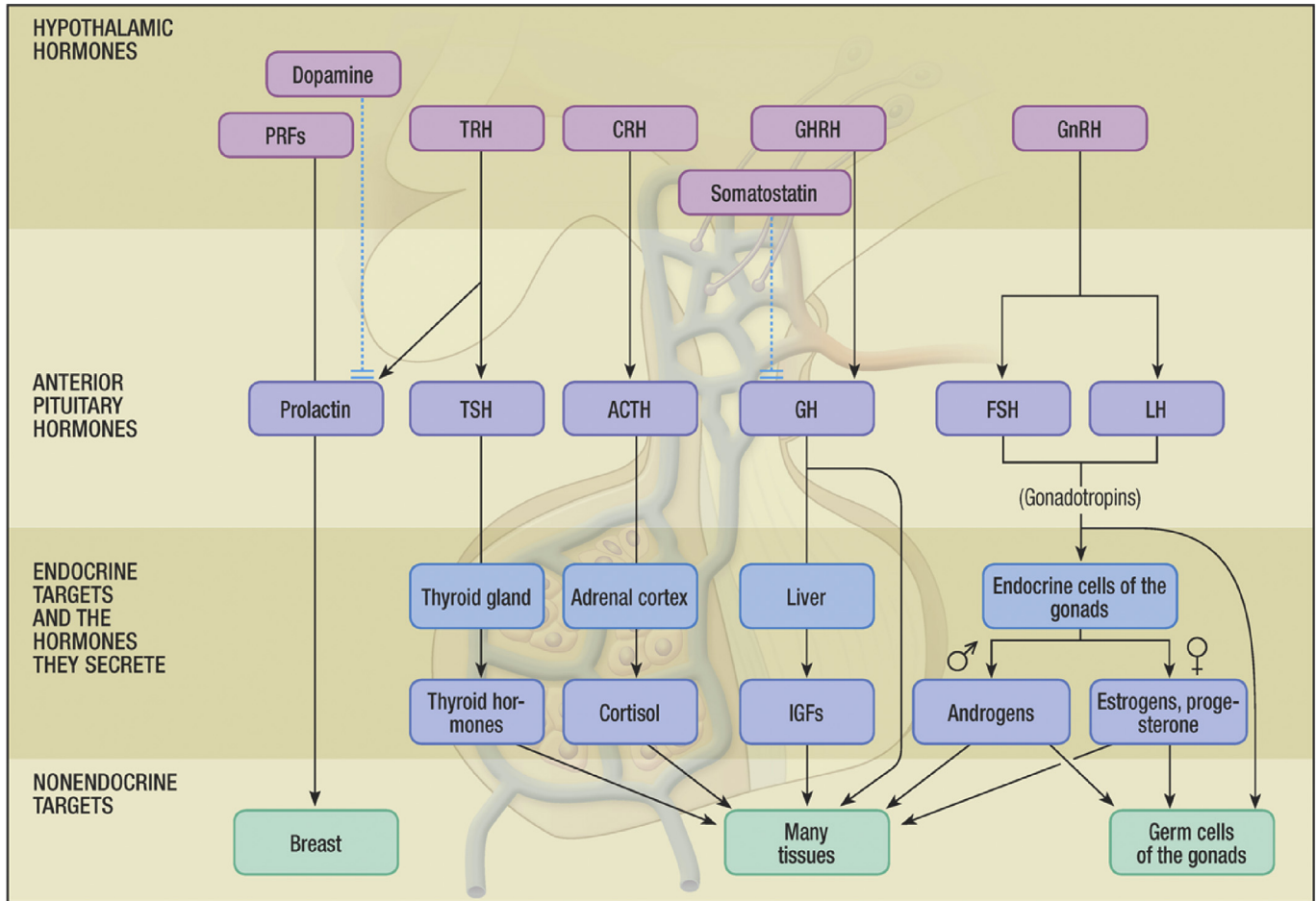
Notably, it appears that GC exert their anti-inflammatory effects more potently in males than in females, suggesting that synthetic GC might not be the most efficacious treatment paradigm for chronic inflammatory diseases in women. Thus, more personalized treatment strategies taking into account sexual dimorphism may need to be considered.

### 3.4. Regulation of the ocular surface and adnexa by hormones from the hypothalamic-pituitary axis

The hypothalamic-pituitary axis is the master regulator of the endocrine system. The hypothalamus processes signals from the central nervous and peripheral endocrine systems, and translates these inputs into signals to the anterior and posterior pituitary (hypophysis) gland. The pituitary then releases hormones that influence multiple tissues, and most endocrine organs, in the body and the hormones that mediate these regulatory functions include prolactin-releasing factor, thyrotropin-releasing hormone, corticotropin-releasing hormone, growth hormone-releasing hormone, gonadotropin-releasing hormone, thyroid-stimulating hormone, growth hormone, adrenocorticotropic hormone, follicle-stimulating hormone, and luteinizing hormone (Fig. 4).

As concerns the eye, hypophyseal hormones exert a significant influence on the ocular surface and adnexa. They are best known to modulate the growth, differentiation and function of the lacrimal gland [360,616,737–740], and to play a direct or indirect (e.g.





**Fig. 4.** Hormones from the hypothalamic-pituitary axis. Abbreviations: PRF – prolactin-releasing factor; TRH – thyrotropin-releasing hormone; CRH – corticotropin-releasing hormone; GHRH – growth hormone-releasing hormone; GnRH – gonadotropin-releasing hormone; TSH - thyrotropin-releasing hormone; GH – growth hormone; ACTH - adrenocorticotropic hormone; FSH - follicle-stimulating hormone; LH - luteinizing hormone.

regulation of sex steroid levels) role in promoting the sexual dimorphism of lacrimal tissue [91,616]. The actions of the hypothalamic-pituitary axis in general, and specific pituitary hormones in particular, on ocular surface and adnexal tissues in health and disease are described in this section. The effects of growth hormone are described in Section 3.5.

#### 3.4.1. Impact of interrupting the hypothalamic-pituitary axis

Most information concerning the impact of the hypothalamic-pituitary axis on the ocular surface and adnexa relates primarily to the lacrimal gland and tear film. These data were obtained in animal studies following hypophysectomy, selective anterior pituitary ablation, or interruption of the hypothalamic-pituitary connection, as well as in dwarf mice with deficient pituitary function. These conditions cause a functional castration and reportedly erase sex-associated differences in the lacrimal gland and induce cytoplasmic vacuolar metamorphosis, nuclear pyknosis, acinar epithelial cell contraction, glandular atrophy, enhanced sulphate uptake, reduced tissue protein, total RNA and mRNA, decreased fluid and protein secretion and a reduced tear volume [12,13,87,91,100,204,360,378,417,616,741–745]. The decline in lacrimal gland weight in hypophysectomized animals (e.g. male rats) seems to be the consequence of an overall reduction in body weight, because the LGW/BW ratios in these animals are typically unchanged [204]. Further, whereas acinar epithelial cells may contract following anterior pituitary disruption, the density of

acinar complexes may increase, but changes can be species specific [204]. The extent of lacrimal gland alterations following interruption of the hypothalamic-pituitary axis is often significantly greater in males than in females [91,743]. In addition, the influence of pituitary dysfunction on lacrimal tissue appears to be attributed to a loss of anterior, but not posterior, lobe hormones [408,410,411,616]. It is likely that the ability of pituitary extracts to increase LGW in guinea pigs [746] is due to hormones from the anterior pituitary. Such extracts also promote the proliferation of human meibomian gland epithelial cells [747]. Indeed, investigators reported that treatment of patients with an anterior pituitary extract attenuated their DED [410,411]. For comparison, the administration of posterior pituitary hormones to rabbits had no impact on lacrimal gland secretion [408].

Of particular interest, many but not all [745] androgen effects on the lacrimal gland are critically dependent upon an intact hypothalamic-pituitary axis. For example, acute or chronic testosterone administration has no consistent influence on the tear volume or protein concentration in orchietomized animals that had undergone anterior pituitary ablation, hypophysectomy or pituitary transplant to the kidney capsule [12,204,412]. Similarly, androgens do not affect the weight, morphology, LGW/BW ratio or fluid secretion of lacrimal glands in pituitary-deficient animals [12,204,412,417,616], and their control of the lacrimal secretory immune system *in vivo* is almost completely inhibited by prior anterior pituitary ablation or hypophysectomy [529]. The

mechanism(s) by which pituitary removal interferes with androgen action on the lacrimal gland may be complex [366,378]. Some of the underlying factors may be the significant decrease in the androgen receptor protein expression in acinar epithelial cell nuclei [97], as well as the significant reduction in androgen-induced transcriptional and post-transcriptional events in lacrimal tissue, following hypophysectomy [91].

However, if the pituitary is transplanted to the kidney capsule, androgen therapy increases the acinar complex area in lacrimal glands of hypophysectomized rats and delays the loss of LGW [204]. Further, treatment of hypophysectomized rats with testosterone and insulin significantly increases the LGW and the LGW/BW ratio [204]. The mechanism(s) underlying these androgen actions remain to be determined.

The impairment of lacrimal gland responses to androgens in pituitary-deficient animals does not represent a generalized lack of tissue responsiveness throughout the body. Testosterone administration to rats without anterior pituitaries induced a 20-fold increase in both the seminal vesicle weight (SVW) and SVW/BW ratio [204]. Thus, the influence of interrupting the hypothalamic-pituitary axis on androgen target organs appears to be site-specific.

### 3.4.2. Effects of hypothalamic-pituitary hormones

Anterior pituitary hormones, either directly or indirectly (e.g. through control of steroid, thyroxine and insulin), modulate the growth, differentiation and secretion of the lacrimal gland [360,410,411,514,616,737–740,742,748–750], as well as meibomian gland function [751]. The effects of growth hormone are described in Section 3.5.

**3.4.2.1. Prolactin.** Investigators have reported that prolactin increases the acinar cell diameter, nuclear size, and lacrimal gland weight of male and female dwarf mice [616], promotes the Na<sup>+</sup>,K<sup>+</sup>-ATPase and cholinergic receptor activities in lacrimal tissues of hypophysectomized female rats [360], and decreases carbachol-induced secretion by acinar epithelial cells [749,752,753]. In addition, prolactin has been proposed to play a minor role in the sexual dimorphism of murine lacrimal glands [748]. However, prolactin has also been demonstrated to exert no effect on the morphology (i.e. in hyperprolactinemic male mice) [748] and weight (i.e. in hypophysectomized male rats) [204] of lacrimal tissue, the synthesis and secretion of proteins by the lacrimal gland [366,752], and the volume of tears [204]. Also, exposure to increased prolactin levels, induced by metoclopramide treatment, leads to a structural disorganization of the mouse lacrimal gland [754].

The origin of lacrimal gland prolactin is not solely the pituitary. Prolactin and its receptor are transcribed and translated in lacrimal gland acinar epithelial cells [750,752,753,755–761]. Prolactin is also secreted by the lacrimal gland into tears [757,762]. It is unclear, though, what factors may modulate the synthesis and secretion of intra-lacrimal prolactin. Treatment with prolactin, prolactin antagonists (i.e. bromocriptine), or estrogen has no effect on lacrimal gland prolactin levels [763], and the administration of cholinergic agonists (i.e. pilocarpine) does not alter the concentration of prolactin secreted by lacrimal tissue [762]. It is possible that lacrimal prolactin could be regulated by androgens, given that the synthesis of this hormone and its receptors are regulated by androgens in other sites [764–767].

In summary, the species-independent role of prolactin in lacrimal tissue and tear film dynamics is unknown. Researchers have found a strong negative correlation between serum prolactin levels and tear function [768]. Considering this hormone's pro-inflammatory actions [766,769] and its proposed role in the pathogenesis of Sjögren syndrome [770,771], it is quite possible that the lacrimal synthesis of prolactin may act to promote autoimmune

disease in the lacrimal gland. Conversely, testosterone's ability to down-regulate the prolactin receptor gene in the lacrimal gland may be one mechanism by which androgens suppress inflammation in this tissue in Sjögren syndrome [351].

With regard to the meibomian gland, investigators have found that women, but not men, with seborrhic MGD have significantly elevated serum levels of prolactin [772]. The reason for this linkage is unknown. Prolactin fragments, in turn, have been reported to inhibit corneal angiogenesis [773].

**3.4.2.2.  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH) and adrenocorticotrophic hormone (ACTH).** The melanocortins  $\alpha$ -MSH and ACTH are involved in the control of constitutive proteins by the lacrimal gland. These hormones have receptors on acinar epithelial cells of the rat lacrimal gland [738,740,774–777] and may stimulate cAMP production and protein release [737,738,740,778,779]. However,  $\alpha$ -MSH and ACTH do not influence the output of all (e.g. regulated) lacrimal gland proteins [366].

When combined with androgens,  $\alpha$ -MSH may increase lacrimal gland weight in orchietomized rats [417]. Androgen treatment also up-regulates the lacrimal gland gene expression for the melanocortin 3 receptor [351], which may enhance the  $\alpha$ -MSH- and ACTH-induced protein secretion [738,740]. In contrast,  $\alpha$ -MSH has no effect on the relative (i.e. LGW/BW ratio) or absolute LGW [204,417], interactions between lacrimal acinar epithelial cells and lymphocytes [780], or the IgA content [741] or volume [204] of tears. Further, ACTH has no effect on acinar cell or nuclear diameters in lacrimal glands of pituitary-deficient mice [616].

Investigators have found that topical  $\alpha$ -MSH application promoted the volume and stability of tears, improved corneal integrity and suppressed ocular surface inflammation in rats with scopolamine-induced DED. These  $\alpha$ -MSH effects could be prevented by pharmacologically blocking either the PKA-CREB or MEK-Erk pathways [781].

Of interest are three additional studies concerning ACTH. First, ACTH may be synthesized by, or accumulate within, myoepithelial cells in the lacrimal gland [755]. Second, circulating ACTH levels have been positively correlated with central corneal thickness [782]. And third, an ACTH insensitivity syndrome (e.g. Allgrove) has been described, which is characterized by adrenal insufficiency, glucocorticoid deficiency and alacrima [783–789]. The reason for the ACTH-cornea association may relate to this peptide hormone's ability to influence corneal regeneration [790] and the mitotic index of corneal epithelium [791]. However, the consequence of lacrimal ACTH synthesis, and the specific cause of the decreased tear output in Allgrove syndrome, remains to be determined.

**3.4.2.3. Thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and vasopressin.** TSH, FSH and LH all increase the weight and differentiation of lacrimal glands in male, but not female, pituitary dwarf mice [616]. The reason for these sex-related differences in hormone action has yet to be clarified. Also to be determined is whether these pituitary hormone effects are direct (e.g. through lacrimal gland receptors), and/or are mediated through the regulation of other hormones (e.g. sex steroids, thyroxine). This question is very relevant for patients with hypothalamic hypogonadism, which was found associated with obstructive MGD in a 35-year-old male patient [792]. This condition is characterized by an impaired pituitary secretion of FSH and LH, leading to hypogonadism and a lack of sex steroid production. The authors of that case report proposed that this lid condition was due to androgen deficiency [792].

TSH receptors have also been identified in the human lacrimal gland [793]. These receptors are thought to be the target for autoantibodies in thyroid-associated ophthalmopathy, and, possibly

through aberrant signal transduction, contribute to the reduced aqueous tear secretion and ocular surface damage in this disorder [793]. TSH levels in the serum of women, but not men, are also increased in seborrhic MGD [772]. The basis for this correlation is not known.

Vasopressin may be synthesized or accumulated by lacrimal gland epithelial cells [794], but the local effect of this hormone is unknown. Similarly, the potential effects of other hypothalamic and pituitary hormones on the ocular surface and adnexa have yet to be identified.

### 3.5. Growth hormone, insulin-like growth factor 1 and insulin regulation of the ocular surface and adnexa

Growth hormone (GH), insulin-like growth factor (IGF-1), as well as insulin are anabolic promoters responsible for mitosis, tissue growth, differentiation and repair. These actions are crucial for the health and functions of lacrimal gland, meibomian gland and ocular surface tissues as detailed below. GH, also called somatotropin, is an anterior pituitary hormone with actions mediated by effects of IGF-1 (also called somatomedin) via endocrine, paracrine and/or autocrine actions. GH, IGF-1 and insulin are involved in metabolism of glucose, amino acids, DNA, lipids and proteins [795–797]. These events are regulated by their respective cell surface receptors, which activate signaling molecules in the cytoplasm, eventually leading to powerful regulation of gene expression that favors cell survival and proliferation [798].

#### 3.5.1. GH, IGF-1 and insulin mechanisms of action, interrelationships, and influence on the ocular surface and adnexa

GH binds to pre-dimerized GH receptors located on cell plasma membranes, leading to activation of intracellular Janus-kinase 2 (JAK2) [799], a kinase that phosphorylates itself as well as the GH receptor [800]. JAK2 further activates Signal Transducers and Activators of Transcription (STATs), which, upon tyrosyl phosphorylation, dimerize and enter the nucleus to regulate the transcription of GH-specific genes including IGF-1 [801]. GH induces cells to secrete IGF-1, which amplifies and complements GH effects, extending its actions, potentially in all cell types. IGF-1 has also paracrine secretion. It has structural and functional similarity with IGF-2 which, however, is not regulated by GH [802]. It is believed that IGF-2 is primarily a growth factor of the fetus whereas IGF-1 acts in postnatal tissues. IGF-2 in relation to corneal wound healing is briefly discussed in Section 3.5.4. IGF-1 signaling is initiated with binding to membrane bound IGF-1R, which phosphorylates and activates insulin receptor substrate (IRS)-1, leading to the cascade of phosphoinositide 3-kinase (PI3K)/Akt pathway activation [803,804], that is an important regulator of cell cycle progression and cell survival.

Insulin exerts its action via binding to insulin receptor (IR), leading to activation of IRS/PI3K/Akt and MAPK pathways [805]. IGF-1 and insulin have molecular similarity and are able to cross-activate each other's receptors, which are also structurally similar, as well as a hybrid IGF-1R/IR [796,806]. However, the cross affinity for the receptor is 100–1000 times lower than that of the specific molecule, depending on cell type [807–809]. In spite of cross-reactivity, IGF-1 and insulin exhibit different actions. Overall, IGF-1 is more efficient in inducing DNA synthesis and mitosis and insulin is more efficient in promoting metabolic events, potentially due to effects on different target cells. GH, IGF-1 and insulin receptors and their respective signaling pathways have been found in lacrimal gland and ocular surface tissues [364,810–815], and there is evidence of influences on tissue development, and wound healing responses.

**3.5.1.1. Cornea.** There is evidence that central corneal thickness (CCT) is decreased in GH deficient children and adults [782,816–818], and that GH treatment for one year increases CCT in GH deficient children [819]. However, others found no significant difference in CCT but increased corneal resistance factor (CRF) and corneal hysteresis (CH) [820]. CCT is also reported to be increased in acromegalic patients [816,821], but others observed no difference in CCT or corneal biomechanical parameters in acromegalic patients compared to age and sex matched controls [822]. High IGF-1 correlates with increased central corneal thickness in polycystic ovarian syndrome patients [823].

Insulin is present in the human tear film and insulin and IGF-1 receptors are found on the human ocular surface [811]. On the other hand, increased IGF-binding protein 3 is found in human tears, which may attenuate IGF-1R signaling in the diabetic cornea [824]. This may contribute to epithelial compromise and the pathogenesis of ocular surface complications reported in diabetes [824]. Insulin has also been found to promote corneal wound healing and may be of therapeutic benefit in delayed corneal wound closure associated with diabetes (see Section 3.5.4).

**3.5.1.2. Meibomian gland.** The GH/IGF-1 axis may positively regulate meibomian gland growth and function. For example, mouse meibomian gland size is positively correlated with GH and IGF-1 levels in a series of transgenic and knockout mouse lines that represent a spectrum of GH/IGF-1 excess, GH/IGF-1 deficiency and GH/IGF-1 absence [118]. Further, the meibomian gland shows normal morphology in GH/IGF-1 excess mice, but increased abnormalities including hyperkeratinized and thickened ducts, acini inserting into duct walls, and poorly differentiated acini in GH/IGF-1 deficient or absent mice [118]. At the cellular level, IGF-1 activates AKT but not ERK pathway, promotes cell proliferation and intracellular lipid accumulation in cultured human meibomian gland epithelial cells, whereas GH does not have a direct effect *in vitro* [751,825]. These data indicate that GH may exert an indirect effect on the meibomian gland via inducing IGF-1, however further research is needed.

Insulin, similar to IGF-1, activates AKT signaling via the IGF-1 receptor and promotes cell proliferation and lipid accumulation in cultured human meibomian gland epithelial cells [826]. Type II diabetes has been associated with MGD in some studies [116,827]. It is plausible that such an association may be mediated by toxicity of high glucose to meibomian gland epithelial cells, reducing IGF-1 receptor levels as well as other insulin and IGF-1 downstream signaling molecules [826].

**3.5.1.3. Lacrimal gland.** Insulin is itself secreted by the lacrimal gland acinar cells and it was demonstrated in rodents that this hormone is locally produced [828,829]. Insulin promotes tissue maintenance and supports constitutive secretion of elements present in the tears, as revealed in lacrimal gland culture studies [364,774,830]. Increasing the concentration of insulin, transferrin and selenium in the culture media of rat lacrimal gland acinar cells, induces secretion of secretory component, a protein that binds and transports IgA [364]. It also increases the synergic secretagogue effect of DHT [364]. In streptozotocin induced animal models of diabetes mellitus (DM), the loss of insulin results in smaller lacrimal glands with altered morphology of the secretory vesicles and intracellular vesicle trafficking, decreased corneal innervation and tear volume, and reduced concentrations of peroxidase in tears and IgA in the lacrimal gland [13,831–834]. The absence of insulin also increases the expression of oxidative markers such as malondialdehyde, peroxidase, and pro-inflammatory cytokines [831,835,

836].

### 3.5.2. GH, IGF-1 and insulin roles in sex-related differences

**3.5.2.1. Systemic sex-related effects of GH, IGF-1 and insulin.** GH is well known to have a different secretion profile in men and women, resulting in vast differences in liver gene expression patterns [837,838]. Sex steroids modulate GH directly and indirectly via IGF-1 [839–841]. Insulin resistance is observed in post-menopausal women receiving oral but not transdermal combined estrogen and progesterone replacement therapy [842]. A possible explanation would be the inverse relationship between insulin binding sites and the levels of estradiol and progesterone during the menstrual cycle of young women and also lower insulin binding levels comparing those phases to young men, suggesting that sex hormones influence the levels of insulin receptor in target tissues (e.g. monocytes) [843]. This is relevant because it may link higher levels of sex hormones to lower action of insulin on the ocular surface and lacrimal gland. This linkage may help explain the stimulus for DED symptoms during the peaks of sex hormones in the follicular and luteal phase of the menstrual cycle and in polycystic ovary syndrome [313,608,842,844,845]. Overall, sex hormones play a significant role in modulating the activities of GH, IGF-1 and insulin.

#### 3.5.2.2. Sex-related effects of GH, IGF-1 and insulin on the ocular adnexa

**3.5.2.2.1. Lacrimal gland.** Neither sex nor the phase of the estrous cycle change insulin receptor levels or early signaling steps in the rat lacrimal gland [73]. The number of androgen receptor-containing cells in lacrimal gland of rats and BALB/c or C57BL/6 mice were higher in male compared to female [97]. Streptozotocin-induced DM did not reduce the number of androgen receptor-containing cells in the rat lacrimal gland [97].

Non-obese diabetic mice (NOD) are widely used in research related to type 1 DM and Sjögren syndrome [846]. This animal model spontaneously created after hyperglycemic sibling inbreeding for several generations, presents a sex biased distribution of manifestations. NOD females are about 80% more frequently affected by DM and sialoadenitis and males are more affected by dacryoadenitis [65,847]. These sex-related differences are attributed to sex hormones [524,848].

Since the early steps of insulin signaling are similar in male and female lacrimal gland the sex hormone-driven changes in insulin, GH or IGF-1 signaling/effects are possibly occurring in later steps or at transcriptional levels of the signaling networks as observed in the modulation of MAP kinase or STAT by sex hormones in cultured cells [849,850]. In support of this hypothesis, testosterone treatment of orchietomized mice altered the expression of insulin receptor-related receptor and insulin-like growth factor binding protein 3 [351]. In meibomian gland immortalized cell cultures, exposure to DHT increased the expression of genes related to insulin signaling [560]. The differences in gene expression in both studies include genes related to development, growth, metabolism, transport and other common actions associated with insulin, IGF-1 and GH, confirming their interactions with sex hormones.

**3.5.2.2.2. Meibomian gland.** Wild type female mice have larger meibomian glands than males, but there is no difference in size between male and female mice that are GH deficient [118]. It is possible that the sexually dimorphic GH effect is responsible, however it is not clear how this interaction of sex and GH affects meibomian gland function or MGD. Both GH and IGF-1 receptor mRNAs are expressed in the mouse [547] and human meibomian gland [110,560], where IGF-1 directly stimulates human meibomian gland epithelial cell proliferation and lipid accumulation [751], and mice show positive correlation in meibomian gland size

with GH/IGF-1 activities [118].

### 3.5.3. Clinical relevance of GH, IGF-1 and insulin in DED

Acromegaly is a disease with overexpression of IGF-1 and consequently also higher secretion of IGF-1 with changes in the body structure and systems. An evaluation of 59 patients with acromegaly compared to 62 age and sex-matched controls revealed a slightly lower tear film breakup time ( $9.1 \pm 3.6$  vs.  $10.7 \pm 2.9$ ,  $p = 0.009$ ), but no differences in Schirmer test or tear film osmolarity, and no clinical differences in DED exams, despite levels of GH and IGF-1 that were on average more than double [851]. Dwarfism, a clinical condition marked by short stature, that is sometimes secondary to GH deficiency, has not been reported to be associated with DED. Gigantism, which is caused by overproduction of GH, has also never been associated with clinical findings of DED.

Pituitary deficiency has been associated with Sjögren syndrome and its clinical manifestations, including DED [852]. The expression of insulin receptor was increased while IGF-1 receptor was decreased, in minor salivary gland (MSG) tissues of Sjögren syndrome patients compared to controls [853]. Interestingly, IGF-1 itself has been found to co-localize with lymphocyte infiltration in Sjögren syndrome salivary glands, suggesting that IGF-1 may be a target of autoimmunity in Sjögren syndrome [854].

Several clinical conditions are associated with insulin resistance and DED or DE symptoms. Among them are polycystic ovary syndrome, pregnancy, anti-androgen therapy and complete androgen insensitivity syndrome [10,540,608,855–857]. Interestingly they also present with changed levels or impaired action of sex hormones, in a complex manner with no clear cut cause-effect relationship with insulin resistance [858,859]. Moreover, DED is the most common side effect of figitumumab, an IGF-1 receptor blocking anti-cancer drug, in healthy human subjects. [860] The development of DED following IGF-1 receptor blockade may be due to disruption of meibomian gland or lacrimal gland function, and/or corneal innervation.

DED is associated with aging, and aging is accompanied by reduced levels of sex hormones and increased insulin resistance [19,861]. Aging in insulin-resistant rats shows increased oxidative stress and impaired vesicular transport, reduced tear flow, and higher levels of pro-inflammatory cytokines and other markers of tissue degeneration in the lacrimal gland, but normal levels of insulin in tears [862–864]. On the other hand, caloric restriction, a strategy known to retard the progress of aging and degenerative diseases in animals, reduced Sjögren syndrome related phenotypes in animal models, via reduction of insulin secretion and action [865–868]. With regard to corneal innervation, IGF-1 treatment not only accelerates corneal nerve regeneration, but also relieves DED in rabbits post LASIK surgery [869].

#### 3.5.3.1. Diabetes and DED

**3.5.3.1.1. Experimental data.** Type I diabetes is known to cause DED [870], which can be secondary to autoimmune destruction of the lacrimal gland due to antigen cross activity with the pancreas [871]. DED secondary to autoimmunity has a similar distribution between the sexes and it is controversial as to whether metabolic factors play a role in type 1 diabetes-induced DED. Several animal studies have shown that insulin can restore lacrimal gland morphology, arguing for a metabolic or hormonal role of lacrimal gland dysfunction in diabetes. In streptozotocin-induced diabetic rats, the lacrimal gland shows reduced number and enlarged size of secretory vesicles, and insulin treatment restores the density of these vesicles [833]. Further, in this rat model of diabetes, changes in morphology, increased numbers of lipofuscin-like inclusions, increased malonaldehyde and total peroxidase activity were observed in the lacrimal gland, and these abnormalities were

removed by insulin treatment [836]. In addition, expression of advanced glycation end products and their receptor, which are related to hyperglycemia, are increased in lacrimal glands of streptozotocin-induced diabetic rats [835]. Therefore even in type I diabetes, lacrimal gland dysfunction has a significant hormonal and metabolic component, plus a possible cross-reactive autoimmune component.

In contrast, type II diabetes associated DE is most likely hormonal or metabolic in nature. This disease, initiated by defective insulin action and hyperglycemia, decreases the microvascular, neural and metabolic integrity of the ocular surface, lacrimal gland and meibomian glands. It is possible that a direct effect of insulin via the IGF-1 receptor, and adverse signaling events caused by high glucose levels, on meibomian gland epithelial cells [826], may be involved in the MGD caused by diabetes.

**3.5.3.1.2. Clinical data.** Clinical ocular surface manifestations of diabetes include lower corneal sensitivity, lower tear film break up time and Schirmer test, epithelial metaplasia, and changes in tear proteins, and are reported to worsen with longer duration of disease and poor glycemic control [872–875]. Tear film osmolarity is higher in DM, compared to other causes of DED, and that may relate to a higher blood osmolarity reflecting a poor glycemic control [876]. This last conclusion is supported by an observation in which individuals with higher blood osmolarity were more prone to DE symptoms [877].

#### 3.5.4. GH, IGF-1 and IGF-2, and insulin on corneal wound healing and neurotrophic keratitis

GH is known to promote skin epithelial wound healing [878–882]. In fact, it is often used off-label to promote skin healing associated with burns and surgery [878]. These observations led investigators to hypothesize that GH may also play a role in corneal epithelial wound healing and/or corneal nerve regeneration [815,883]. GH has been shown to promote corneal epithelial cell migration independent of IGF-1 *in vitro* [815]. More studies need to be performed to confirm positive effect of GH on corneal wound healing and/or nerve regeneration *in vivo*. If true, this may be beneficial in the treatment of corneal epithelial defects and/or neurotrophic keratitis associated with severe DED.

IGF-1 has been studied more extensively in terms of corneal wound healing, and found to promote corneal wound healing at multiple levels. First, IGF-1 promotes the proliferation and migration of corneal epithelial cells and fibroblasts [884–888], differentiation of limbal stem cells [889,890], and the proliferation of corneal endothelial cells *in vitro* [891–893]. Second, IGF-1 accelerates corneal epithelial migration and wound healing in organ culture [894–896] and animal models [897–900]. Third, IGF-1 also preserves corneal nerves in diabetic animals [890] and animals undergoing LASIK [869]. Lastly, in some human studies, IGF-1 has been found to prevent superficial punctate keratopathy in diabetic patients post cataract surgery [901], and accelerate reepithelialization in patients with neurotrophic keratopathy [902–904]. A summary of the findings of the role of IGF-1 in corneal wound healing is provided in Table 8 [869,884–913]. Insulin has been found to duplicate IGF-1 in promoting corneal wound healing in cell culture, organ culture and diabetic animal models of corneal wound healing. These findings are summarized in Table 9 [911,914–930].

Systemic replacement and/or usage of topical insulin treatment can reverse the signs of DED and wound healing defects in animal models of DM [836,920,931,932]. Autologous serum, which also contains insulin and growth factors has been used to attenuate the signs and symptoms of severe DE and delayed wound healing not just in DM, but also in several conditions associated with DE [933–935]. (See also TFOS DEWS II Management and Therapy Report) [936]. Different formulas and delivery systems of insulin and IGF-1 eye drops have been developed for DE treatment and a novel delivery system has been proposed to improve stability and the target tissue concentration of insulin [937–939].

IGF-2 has been demonstrated to act via IGF-1R in human embryonic corneal endothelial cells and stimulate their proliferation [940]. IGF-2 is also present in bovine corneal stroma, and stimulates proliferation of cultured keratocytes [941]. Similar to IGF-1 and insulin, IGF-2 also activates AKT signaling and promotes cell proliferation in cultured human corneal epithelial cells [915]. IGF-2R may play an active role in corneal wound healing. For example, IGF2R protein expression is increased during corneal wound healing in mouse corneas and IGF2R regulates human corneal fibroblast

**Table 8**  
Summary of IGF-1 effects on corneal wound healing.

Model used	Epithelial cells/limbal stem cells (LSC)	Stroma/keratocytes	Endothelial cells	Innervation
Cell culture/signaling	<ul style="list-style-type: none"> <li>• ↑ LSC differentiation [889].</li> <li>• ↑ epithelial proliferation and migration, activating AKT and ERK [905].</li> <li>• IGF-1R [906] and IGF-1R/IR hybrid nuclear localization [907].</li> <li>• ↑ laminin-5 and beta-1 integrin [908]</li> <li>• PKC and tyrosine kinase [909]</li> </ul>	<ul style="list-style-type: none"> <li>• ↑ human corneal fibroblast proliferation and collagen synthesis [884–887,911].</li> <li>• Epi releases IGF-1 to act on stroma [912]</li> <li>• ↑ Keratocyte migration [888]</li> </ul>	<ul style="list-style-type: none"> <li>• ↑ rabbit endothelial cell proliferation via IRS-1 [893]</li> <li>• ↑ Bovine endothelial cell proliferation [891]</li> <li>• No effect in cat endothelial cells [913]</li> </ul>	
Organ culture	+Substance P: ↑ epithelial migration and healing [894–896]		↑ cell proliferation in human embryonic endothelial and stromal culture [892]	
Animal model	<ul style="list-style-type: none"> <li>• +Substance P: ↑ healing rat models of neurotrophic keratopathy [897,898] and diabetes [899] and rabbits [900]</li> <li>• Preserves limbal stem cells in type II diabetic mice [890]</li> <li>• No effect in a rat model of galactosemia [910]</li> </ul>			↑ nerves in type II diabetic mice [890], LASIK rabbits [869]
Human study	+Substance P: Prevents SPK in diabetic patients post cataract surgery [901], accelerates reepithelialization in patients with neurotrophic keratopathy [902–904]			

**Table 9**

Summary of insulin actions on corneal wound healing.

Model used	Epithelial cells	Stroma/keratocytes	Endothelial cells	Innervation
Cell culture/signaling	<ul style="list-style-type: none"> <li>• ↑ wound healing in the corneal epithelium by activating PI3K/AKT, ERK and EGFR [914]</li> <li>• ↑ proliferation and anti-apoptotic [915]</li> <li>• ↑ migration but not proliferation [916]</li> </ul>	↑ bovine keratocyte matrix secretion, collagen synthesis [911,923,924]	<ul style="list-style-type: none"> <li>• ↑ the Na, K-ATPase activity and pump function of cultured corneal endothelial cells, mediated by PKC [927]</li> <li>• ↑ bovine and human endothelial cell proliferation [928,929]</li> </ul>	
Organ culture	↑ proteolysis in diabetic corneas [917]			
Animal model	↑ Healing in streptozotocin rats [918–920]			Prevent nerve loss in streptozotocin-induced diabetic mice [930]
Human study	<ul style="list-style-type: none"> <li>• Insulin receptor present on human ocular surface tissue in healthy and diabetic patients [921]</li> <li>• ↑ auto-fluorescence and ↓ corneal sensitivity in type I diabetic patients [922]</li> </ul>	Topical insulin not toxic to ocular surface [925,926]		

to myofibroblast differentiation [942]. IGF-2 also showed elevated expression after corneal injury and may facilitate limbal stem cell differentiation in corneal wound repair in a mouse model of mechanical cornea injury [943].

### 3.6. Thyroid hormone regulation of the ocular surface and adnexa

The thyroid gland secretes two hormones, triiodothyronine (T3) and thyroxine (T4). Ocular and adnexal tissues are targets of thyroid hormones (TH), which have anabolic effects and promote lacrimal gland and other exocrine gland activity [944–947]. Thyroid gland diseases or thyroid hormone (TH) imbalance have negative effects on the lacrimal gland, tear film and ocular surface [948,949]. The causes may be lower hormone inputs to the ocular and adnexa tissues, contiguous inflammatory disease, or higher exposure of the ocular surface due to eyelid wide opening [950–953]. Specifically, Graves' ophthalmopathy, in its early phase is 5-fold more frequently associated with DED than healthy controls. The clinical findings observed in this recent report were lower Schirmer test, lower TBUT with fluorescein, and lower corneal sensitivity, with or without exophthalmos [954].

#### 3.6.1. Thyroid hormone influence and mechanism of action

TH promote lipid and protein synthesis, tissue growth and differentiation [946]. The lacrimal gland and ocular surface epithelia have nuclear receptors for thyroid hormones and therefore are target organs for those hormones. Decreases of T3 and T4 induce hypotrophy of the lacrimal gland, cornea epithelia metaplasia and reduced tear flow [399,944,955].

TH work primarily through nuclear receptors, which promote the expression of specific genes. The two isoforms of the thyroid hormone receptors, alpha and beta, are further divided in types 1 and 2. They are differently expressed in different tissues [956]. Thyroid hormone receptor beta-1 is expressed in rat lacrimal gland and hormone reduction for ten weeks induces its up-regulation in this tissue [944]. TH are well known to influence lipolysis and lipogenesis; their deficiency causes hypercholesterolemia and reduction of lipid secretion by sebaceous glands [946,957], suggesting that TH may also exert influence on the meibomian glands.

#### 3.6.2. Thyroid hormone role in sex-related differences

The frequency of autoimmune thyroid diseases, Hashimoto's

thyroiditis and Graves' disease are several times higher in females than males [958]. Moreover, Sjögren syndrome, a disease ten times more frequent in females is commonly associated with thyroid-associated diseases [948,951]. These events taken together indicate that female sex and/or female sex hormones predispose to inflammatory events in the thyroid gland and related target tissues. In addition, TH imbalance may contribute to the development of autoimmune diseases.

#### 3.6.3. Clinical relevance of thyroid hormone action in DED

Several diseases related to the thyroid gland and its hormones may affect the ocular surface and induce DED. The three major mechanisms are mechanical (proptosis associated with Graves' disease), autoimmunity that extends to ocular and adnexal tissues; and TH loss in conditions related to iodine scarcity, thyroid radiotherapy, thyroid gland ablation and poor hormone replacement in different diseases.

Thyroid-associated ophthalmopathy features DED manifestations, including symptoms, low tear secretion, low TBUT, punctate keratitis, rose bengal staining, higher tear osmolarity and MGD [793,959]. A challenging aspect is that no single test is capable of distinguishing patients with DED due to Graves' ophthalmopathy in a population setting [876]. A recent study using proteomics to compare the profile of tears from patients with Thyroid-associated orbitopathy (TAO), DED patients without TAO and healthy controls revealed that a similar profile in TAO with or without DED, compared to DED and control groups [466]. Although the work did not clearly define the criteria to label DED individuals or the possible causes of DED in the non TAO group, it indicated a higher expression of pro-inflammatory proteins in both TAO groups, suggesting possible biomarkers and unique mechanisms of disease in DED caused by TAO [960].

## 4. Gender, health, and DED

### 4.1. Sex and gender as interrelated distinct characteristics

Animal studies reveal sex-based differences that are rooted in biology and appear to affect risk for DED and could shed light on the mechanisms behind the disease and its presentation [89,98,263,265,527,671]. For example, Zylberberg et al. [671] found increased levels of the pro-MMPs-2 and -9 in cell cultures obtained from

female rabbits' lacrimal glands that were exposed to estradiol but not to DHT. Both of these MMPs are found in increased concentrations in tear fluids from patients with DED, suggesting that these MMPs may be implicated in the pathogenesis of this disease. In addition, Seamon et al. [98] demonstrated reduced levels of tear lipocalin in ovariectomized rabbits, adding to the evidence linking reduced levels of sex steroids with DED—a finding that might help explain why postmenopausal women are at increased risk of DED (see Section 2.2.3).

When considering health and disease and the provision of health care, gender—the socially constructed differences between men and women that give rise to conceptions of masculinity and femininity—must also be taken into account [961]. Various factors related to sex and gender place women at heightened risk for DED and make them vulnerable to disparities in care and outcomes. The literature abounds with examples of gender-based health disparities in access to care, care-seeking behavior (particularly in women in developed countries), communication with health care providers, service utilization, and health outcomes around the world, and these examples may likewise apply in the case of DED. Studies, for example, have documented disparities in colorectal cancer screening [962,963], access to screening and treatment for HIV infection [964,965], quality of life for epilepsy patients [966,967], referrals for cardiac rehabilitation [968], access to and care-seeking behavior for mental health services [969,970], quality of life and long-term outcomes of stroke survivors [971], and care provided for type 2 diabetes and in lower-extremity amputations among patients with diabetes [972,973].

According to Schiebinger and Stefanick [974] gender can be broken down into “gender identity” (how individuals and groups perceive and present themselves), “gender norms” (unspoken rules in the family, workplace, institutional, or global culture that influence individual attitudes and behaviors), and “gender relations” (the power relations between individuals of different gender identities). The many known determinants of women's eye health disparities include uneven access to care and treatment due to socioeconomic factors; attitudes and behaviors about preventive care; gender-based differences in health-seeking behaviors; age [33]. That biological age is a key risk factor for eye and vision problems, including DED, contributes to the notion that women, who live longer than men on average, also suffer significant disability from chronic vision conditions that predominate in older people [33]. In addition, many autoimmune diseases are more prevalent in women than in men [33]. Certain of these diseases (e.g. systemic lupus erythematosus, rheumatoid arthritis) are associated with DED (see Section 2.2.3.).

#### 4.2. Gendered behaviors can lead to gender differences in eye conditions

Trachoma and onchocerciasis, the first and second leading causes of infectious blindness in the world, are classic examples of gender-based health issues. In the developing world, where trachoma is common, women are three times more likely than men to be blinded by trachoma, due to the influence of gender-defined roles for women [975]. Women have more physical contact with people who are infected, putting them at greater risk of exposure. Women are also less likely to seek and receive treatment for trachoma [33]. Onchocerciasis in contrast, disproportionately affects men compared to women. This imbalance is due to the influence of gender roles as well, with men in endemic areas spending more time than women in aquatic environments, such as polluted rivers, where the parasitic disease spreads via an insect vector [33].

Gendered behaviors also affect risk for DED in a significant way, as women may experience heightened attention to their cosmetic appearance, including the wearing of spectacles. For example, in certain countries more women wear contact lenses than men, and contact lens wear is associated with a heightened risk for DED stemming from lens use [976]. Nichols and Sinnott found that women who wore contact lenses were more likely to have DED than were men, with 40% of the men and 62% of the women in the study classified as having DED ( $P < 0.0001$ ) [977]. Women are also more likely to undergo laser refractive surgery [978], which is associated with an elevated risk of developing DED [979–982]. In another study, the combination of oral contraceptive pill use and contact lens wear appeared to increase the severity of DED symptoms in young women [58,699,983].

#### 4.3. Gender concordance between patient and care provider adds another dimension

A substantial body of literature addresses the influence of gender on the interactions between patients and their care providers. It has been postulated that the gender composition of the patient–clinician dyad could affect communication, shared decision-making, and other aspects of health care, but the answer is still not clear. For example, a patient-level meta-analysis by Wyatt et al. [984] across 7 clinical trials (775 clinical encounters) found no statistically significant interaction between clinician–patient gender mix and decisional conflict, satisfaction with the clinical encounter, or patient engagement. A borderline significant interaction was observed only for increased concordance between stated decision and action taken, for encounters involving clinicians who were women and patients who were men ( $p = 0.05$ ). All other gender dyads showed decreased concordance (6% fewer concordant encounters for same-gender, 16% fewer concordant encounters for clinicians who were men/patients who were women) [984]. A systematic review of the literature undertaken by Deepmala et al. [985] found that 10 of 12 studies analyzed provided evidence that provider characteristics, including age, sex/gender, experience, and specialty, as well as the interplay between provider and patient characteristics are important variables in pain management with analgesics.

#### 4.4. Sex and gender influence the experience and treatment of pain

Pain is a hallmark of DED. Surveys of epidemiologic and laboratory data as well as electronic medical records provide strong evidence for clinical and experimental sex and gender differences in pain [986,987]. Many studies show that men have higher pain thresholds than women. This relationship holds true even for children and adolescents [988,989]. Various explanations for this phenomenon have been given, ranging from experiential and sociocultural gender differences in pain experience between men and women to hormonally and genetically driven sex differences in brain neurochemistry [986]. Having lower pain thresholds and tolerances (i.e. greater sensitivity to pain) leaves women at particularly high risk of being undertreated for pain [990]. Health care providers should take into account the role of gender when assessing their patients' complaints of pain because men and women differ in their perception of pain, their tendency to complain of pain, and their methods for coping with pain [991,992].

Animal studies can help distinguish the relative contributions of biological sex and gender to pain and pain attenuation. Many studies in animal models have demonstrated sex-specific differences in responses to pain and pain attenuation. In a study of rats,

Liu et al. [993] found a striking interdependence of sex and pain type that determines the manifestation of antinociception (reduction of sensitivity to painful stimulus) mediated by Dyn protein and its associated kappa opioid receptor (KOR). Both sex and pain type are important determinants of Dyn/KOR antinociception; neither variable acts independently of the other. Another group reported sex-specific differences in pain response by dopamine in the bed nucleus of the stria terminalis in rats [994,995]. Yet another study revealed increased heat sensitivity and decreased cold sensitivity for female rats, but not males that underwent injections of quisqualic acid into the thoracic gray matter or sham operations. This selective effect is indicative of altered sympathetic activation by the thoracic injections. The effect of sham surgery suggests that female rats are vulnerable to ischemic injury during exposure and manipulation of the spinal cord [996]. One study examining chronic pain among the elderly found that women tended to adopt more psychologic approaches, such as acceptance and ignoring to relieve pain, than men [997]. In a study of nearly 800 men and women with fibromyalgia syndrome, Racine et al. [991] found that men were more likely to view pain as evidence of harm, and they were also more likely than women to avoid activity as a way to cope. Curtailing activity as a pain-coping strategy appears to be detrimental to men's quality of life [998].

Moreover, the role played by care providers' gender in pain management cannot be ignored. A study involving 310 general practitioners revealed that evidence of pathology had a larger effect on referrals of low-back pain patients to psychology/psychiatry by physicians who were men than those who were women. Also, the gender of the clinician moderated the pain judgments that accounted for the effect of pathology findings and pain behaviors on prescribing patterns [999]. For example, physicians' gender had a significant impact on pain management decisions in patients with low back pain, according to a review of 186 medical records [1000].

Care providers may view subjective complaints differently than objective tests. In cases of DED, this is problematic because symptom complaints do not correlate well with ocular findings. Management of DED symptoms is complex, and health care providers need to consider a patient's holistic picture, rather than simply treating ocular signs. For example, Vehof et al. [293] conducted a cross-sectional study of 1622 twin volunteers, all of whom were women, ranging in age from 20 to 83. A total of 438 (27.0%) were categorized as having DED. Women with the disease had significantly greater pain sensitivity, lower pain tolerance, and more pain symptoms than those without DED, strengthening the evidence of associations between the severity of tear insufficiency, cell damage, and psychological factors.

## 5. Recommendations for future research related to sex, gender, hormones and DED

DED can cause significant pain, but little is known about factors contributing to symptoms of DED, given the poor correlation between these symptoms and objective signs at the ocular surface [274,293]. The hope is that we can identify better ways to predict risk for DED and develop novel therapies to alleviate this condition with more targeted, mechanistic approaches instead of relying on nonspecific symptom relief. Sex, gender and hormones exert a significant influence on the ocular surface and adnexa, and play a significant role in the pathogenesis of aqueous-deficient and evaporative DED. However, further studies are required to clarify the precise nature, extent, and mechanisms of these sex, endocrine and gender effects on the eye in health and disease. Such studies need to:

- Use the terms sex and gender consistently and correctly across scientific disciplines, in order to promote the accurate assessment, measurement, and reporting of differences between men and women;
- Conduct more epidemiological studies on the prevalence of DED by using both sign and symptom data;
- Use the term sex in most studies of nonhuman animals;
- Include sex as a variable in basic and clinical research, and take donor sex into account in experiments with cultured cells;
- Select animal models for research that mirror human sex differences and are relevant for DED;
- Evaluate natural genetic variability, disorders of sex differentiation, reproductive status and environmental influences to gain a better understanding of human DED;
- Elucidate the roles of the sex chromosome complement (e.g. parent-of-origin effects, X-inactivation, and genes in the non-recombining region of the Y chromosome), sex-specific autosomal factors and epigenetics (e.g. miRNAs, DNA methylation and acetylation, and histone modifications), as well as the microbiome, in mediating sex-related differences;
- Develop systems that identify and differentiate the effects of genes from those of hormones;
- Determine the processes involved in the sex steroid, hypothalamic-pituitary hormone, glucocorticoid, insulin, IGF-1 and thyroid hormone regulation of ocular surface tissues, and how they contribute to the sex-related differences in DED;
- Perform clinical studies to determine whether a sexual dimorphism exists in the response to topical GCs for the treatment of inflammatory ocular surface disorders;
- Use functional neuroimaging (e.g. positron emission tomography, fMRI) to evaluate sex-related differences in the pain of DED, as well as in experimentally-induced pain stimuli to the ocular surfaces of healthy subjects;
- Develop innovative human experimental models that better mimic clinical pain in DED;
- Determine whether sex differences exist in the pattern of innervation, the capacity to release neurotransmitters, and the sensitivity to neural stimulation, in ocular surface and adnexal tissues;
- Determine whether sex differences are present in the levels of tear film biomarkers in health and disease, and whether these measures could be used diagnostically;
- Assess whether diagnostic tests and management of DED should be different in men and women;
- Examine the utility of local (i.e. intracrine) hormone measurements for the diagnosis of DED;
- Conduct clinical studies to determine the extent to which MGD, as defined by using meibomian gland diagnostic tests, shows sex-related differences;
- Determine whether the sex difference in DED lessens with more advanced age, becoming more similar among women and men;
- Communicate clearly about the role of sex and gender influences in the arenas of DED research, patient care, and health and science policy;
- Determine whether the use of cosmetics contributes to the gender-related differences in the prevalence of dry eye disease;
- Ensure adequate participation of women in clinical trials, and analyze data by sex/gender to determine differences in response to treatment.

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