Local synthesis of sex hormones: Are there consequences for the ocular surface and dry eye? Intracrinology and the ocular surface

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ABSTRACT

Sex hormones are associated with the physiology and pathophysiology of almost all organs in body, as well as most diseases. Interest in the associations between sex hormones and ocular tissues has increased in recent years. Androgens may have a positive effect on dry eye, whereas the effects of estrogen on ocular conditions remain unclear. Intracrinology, the local synthesis and metabolism of hormones which is unique to humans, is of relevance to the eye and may help to explain why studies of the relationship between estrogens and dry eye signs and symptoms are inconclusive. Knowledge of the pathways of hormone formation and metabolism is crucial to understanding the pathogenesis of ocular disease including dry eye. This review examines the mechanisms of steroidal sex hormone biosynthesis and reviews the significance of locally produced sex hormones, with a focus on ocular surface tissues. Much of the current literature is based on animal studies, which may not be transferable to humans due to the absence of intracrine production in animals. A large proportion of the human studies investigate systemic hormones levels rather than local levels. There is subsequently a need for additional studies to provide a better understanding of the local production of sex hormones within the human eye and ocular surface and to clarify the relationships between ocular levels of sex hormones and conditions including dry eye.

KEYWORDS intracrinology, androgens, estrogens, dry eye disease, MGD, ocular surface

Abbreviations are printed in **boldface** where they first appear with their definitions.

Literature review: methods

A review was conducted to assess whether the local synthesis of sex hormones has consequences for the physiology and function of the ocular surface and a subsequent impact on dry eye disease. Relevant articles were identified, published to July 2017, through searches in PubMed, Scopus and Medline databases as well as through the reference lists of identified publications. Searches were performed for meeting abstracts for the American Academy of Optometry (AAO), the American Academy of Ophthalmology (AAO), the Tear Film, and Ocular Surface Society (TFOS) and the Association for Research in Vision and Ophthalmology (ARVO). Patent searches were also carried out.

Search terms included: *sex hormones, sex steroids, intracrinology, estradiol, estrogen, testosterone, androgen and steroidogenesis.* Additional search terms for each section include: Section II: *endocrinology*; III [A] *meibomian gland, lacrimal gland, cornea and conjunctiva, receptors, mRNA, gene regulation, [B] mass spectrometry, tears, meibum, blood, human, [C] ocular impact, hormone replacement therapy, oral contraception, estrogen therapy, androgen therapy, treatment, topical, dry eye, keratoconjunctivitis sicca, MGD, symptoms, TBUT, Schirmer, function;* [B] .

I. INTRODUCTION: SEX AND DRY EYE

Epidemiology

Approximately 3.2 million women and 1.68 million men in the United States suffer from severe dry eye symptoms or clinically diagnosed dry eye, with millions more experiencing less intense symptoms. [1,2] Large epidemiological studies have identified female sex and older age as risk factors for the development of dry eye. [1–10] The risk of experiencing dry eye appears to increase over two times with menopause.[11]

Pathophysiology

Dry eye occurs when the tear film is compromised, either by reduced aqueous production or increased evaporation: tear quality and stability is thus impaired. The pathogenesis of dry eye is complex and as yet not completely understood. Its aetiology is multifactorial; in addition to inflammatory processes and neural feedback mechanisms, there is also strong evidence for a hormone-mediated contribution.[9,12]

Treatment of dry eye

Medications, such as anti-inflammatory and anti-biotic agents, can be used for treating processes involved in dry eye, including reducing the presence of inflammatory mediators or pathogens on the ocular surface.[13] However, many traditional treatments for dry eye disease are only palliative, including artificial lubricants or retention plugs, aimed at increasing the volume of tears on the ocular surface. A treatment directed at the underlying cause has the potential to provide effective relief to the millions of people worldwide who suffer from dry eye disease; this is not currently possible due to the current uncertainty of the pathophysiology of dry eye.

Sex-related differences

Sex-related differences are found in almost every cell and tissue in the body, thus it is rational to expect that sex-differences also occur in ocular tissues. [14] Vernal keratoconjunctivitis (VKC) is a well-documented example of an ocular surface disease which shows significant sex-related prevalence, with over three quarters of patients being male.[15] VKC also shows a tendency to resolve around puberty, in both males and females, which suggests a hormonal effect on VKC. [15] In addition, the number of estrogen and progesterone receptors in the epithelium and sub epithelium of the tarsal

and bulbar conjunctiva has been shown to increase with disease duration, which suggests a possible relationship between these hormones and VKC. [16]

This review examines the mechanisms of steroidal sex hormone biosynthesis and considers the implications of local hormone production on investigations of hormone effects on dry eye.

II. SEX HORMONES

Sex hormones, including androgens and estrogens, are steroids responsible for some of the most profound changes which occur to the body. Sex hormones are associated with the physiology and pathophysiology of almost all organs, as well as most diseases. Figure 1 shows the steroidogenic pathways.

A. Sex hormone biosynthesis

1. Synthesis of circulating sex hormones

Androgens and estrogens are biologically active in both sexes and it is erroneous to associate estrogens with female and androgens exclusively with male sex. In men the testes provide a near continuous supply of androgens and small amounts of estrogens through a male's lifetime.[17] In women the ovaries secrete estrogens, progesterone and androgens. However at menopause ovarian secretion of estrogen and progesterone stops.[18] Women undergo a slow decline in testosterone with age, which doesn't appear to be associated with the final menstrual period.[19]

In adult men, blood testosterone levels are 100-150x the level of estradiol.[20] In pre-menopausal adult women, during the early follicular phase of the menstrual cycle (when estrogen and progesterone levels are low), circulating testosterone levels are 2-3x that of estradiol, with the ratio increasing post-menopause (>5years) to 80 times the level of estradiol. [21]

2. Intracrinology: Synthesis of sex hormones in peripheral tissue

As well as being secreted by the gonads and adrenals, sex hormones are produced in peripheral tissues throughout the body from circulating precursors of adrenal or gonadal origin.[22] Intracrinology was pioneered as a new branch of endocrinology by Ferdinand Labrie, in the late 1980's, describing the mechanisms of the synthesis of

active hormones in peripheral tissues from DHEA and its sulfate DHEA-S (see FIG 1). During intracrinology, hormones exert their action within the same cells in which they are synthesized and in which they are metabolized before being released into circulation as inactive compounds.[22] The intracellular inactivation of sex hormones prior to release into extracellular space protects neighbouring tissues from the action of sex hormone thus avoiding possible adverse effects of their systemic circulation.[23]

A key difference between endocrinology and intracrinology is that endocrine organs, such as the ovaries, disperse synthesized sex hormones via general circulation to all bodily tissues. Conversely, intracrinology allows individual tissues to synthesize the required amount of estrogens and androgens without releasing significant amounts of biologically active hormones into circulation.[24,25] As the only animal species with adrenals that secrete large amounts of DHEA and dehydroepiandrosterone sulfate (**DHEA-S**), humans and other primates are unique in their ability to produce hormones locally.[23] DHEA and DHEA-S are received by the local cells from circulation then converted into androstenedione (4**-dione**) and subsequently into androgens and estrogens (FIG 1) [26] at levels required by the specific cells.[24] There is an abundance of DHEA in serum, with serum levels of DHEA being about 10 times higher than testosterone levels, and 500 times higher than estradiol levels.[27]

In humans, DHEA levels in circulation reach maximum levels between age 20-30 years, decreasing by 80% by the age of 70 in males and females.[28],[29] This great reduction in adrenal secretion of DHEA and DHEA-S results in a large fall in the formation of androgens and estrogens in peripheral tissues with age.[30] If tear producing tissues are presumed to produce sex hormones locally by intracrinology, this drop in DHEA and DHEA-S could be a contributing factor to the increase of dry eye with age.[3]

Celec describes DHEA as a “human molecule” because levels in humans are so much higher than in animal species.[31] The adrenals of rats and other animals do not secrete significant amounts of DHEA required for synthesis of sex hormones by intracrinology.[32] Consequently rodents, who secrete sex steroids solely from the gonads, are very different to humans. Hence as animals lack the ability to make hormones locally, caution should be taken when applying rodent/animal models to human models. As a result, this review focusses on human studies.

III. SIGNIFICANCE OF INTRACRINOLOGY

Measuring testosterone and DHT concentrations in serum indicates testicular function, in men, or ovarian function, in women, and does not include local intracrine production. Almost all androgens in women are made in peripheral tissues. The total androgen pool in both men and women can be better estimated by the serum concentrations of androgen metabolites: androsterone glucuronide (**ADT-G**), 3α-diol-G and 3β-diol-G.[33] In men it is estimated that 30-50% of total androgens are synthesized locally from inactive adrenal precursors.[34] Using the sum of these androgen metabolites in blood, androgen production in post-menopausal women has been calculated to be over two thirds of that of men, much higher than previously thought, [35] yet their serum testosterone concentration is only 3% that of men (15ng/dL in women, 461ng/dL in men).[20,21] Therefore, measuring metabolite levels rather than levels of active androgens provides a more accurate indication of the amount of active androgens within peripheral tissues.

Estrogens produced by intracrine processes are also important. Labrie estimates that pre-menopause, 75% of estrogens are made in peripheral tissues by intracrinology, with this increasing to 100% after menopause (as depicted in Figure 2).[22,26,33] Although the percentage of estrogens being produced by intracrinology increases post-menopause, the total estrogen produced is lower compared with pre-menopause where there are both endocrine and intracrine sources. To achieve a better estimation of the amount of estrogens in the human body many published studies measure estrogen metabolites. These include, but are not limited to, hydroxylated metabolites such as 2-hydroxyestrone, 2-hydroxyestradiol, 16α-hydroxyestrone and estriol [36] or sulphated metabolites.[37]

Intracrine synthesis of sex hormones is vital for maintaining normal function in humans. Peripheral tissues require access to high circulating levels of DHEA and to intracrine enzymes, including aromatase, 5α-reductase, 17β-HSD and 3β-HSD, to enable biosynthesis of estrogens and/or androgens.[33,38] All human tissues, except the endometrium, possess these enzymes (reviewed by Labrie)[24] thus intracrinology allows circulating levels of estrogens to remain sub threshold post-menopause. This avoids stimulation of the endometrium, whilst allowing tissues to create their own estrogens as needed.

A. Sex hormones and the ocular surface

Human ocular surface tissues, like other peripheral tissues, are thought to make sex hormones at levels required by each tissue, by intracrine processes.[22] It is proposed that circulating sex hormones and metabolites are supplied to, and removed from, the ocular surface in a similar way to other nutrients: via diffusion into and from the tear fluid, and blood vessels in the conjunctiva and other ocular tissues. During intracrinology, these sex hormones are used within the cells in which they are synthesised, with minimal release of active hormone out of the tissue. The metabolites are then released into circulation and may also be released into the tears.

Table 1: Summary of intracrine machinery identified in human ocular surface tissue.

Intracrine machinery	Cornea	Conjunctiva	Meibomian gland	Lacrimal gland
Receptor				
AR	+ ([39],[40])	+ ([39])	+ ([39,41,42],)	+ ([39])
ER	+ ([40])	+ ([43])	+ ([41,44])	+ ([45])
PR	+ ([40])	+ ([43])	+ ([41,44])	+ ([45])
AR mRNA	+ ([46],[40])	+ ([46])	+ ([46])	+ ([46])
ER mRNA	+ ([46],[40])	+ ([46],[43],[47])	+ ([46])	+ ([46],[47])
PR mRNA	+ ([46],[40])	+ ([46],[43])	+ ([46])	+ ([46])
Enzyme mRNA				
5α-reductase	+ ([39])	+ ([39])	+ ([39])	+ ([39])
steroid sulfatase **3β- HSD (Type 1)** **17B-HSD (Type1+3)** **aromatase**	+ ([48])	+ ([48])	+ ([48])	+ ([48])
glucuronosyltransferase				

+ refers to the finding of the intracrine machinery in the ocular tissue.

Abbreviations: AR: androgen receptors; ER: estrogen receptors; PR: progesterone receptors; LG: lacrimal gland; MG: meibomian gland; mRNA: messenger RNA, HSD: hydroxysteroid dehydrogenase

1. Steroidogenic enzymes in human ocular surface tissues

The transformation of adrenal precursor steroids, DHEA and DHEA-S, into androgens or estrogens relies on the expression of steroidogenic enzymes in peripheral tissues. [30] Steroid sulfatase, aromatase, glucuronosyltransferase, 3β-HSD-Δ-5Δ4-isomerase type 1, 17β-HSD types 1 and 3, and 5α-reductase types 1 and 2 are all required for the conversion of DHEA-S to androgens and estrogens, and then for metabolism into their inactive glucoronate and sulfated forms (FIG 1).[24] mRNAs for all of the above

steroidogenic enzymes have been found in human lacrimal glands and meibomian glands, as well as in corneal and conjunctival epithelial cells[39,48] (Table 2). This suggests that tear producing tissues are able to produce sex hormones by intracrinology.

2. Possible local action of sex hormones produced by intracrine processes

Sex steroid receptor mRNAs have been found in human ocular surface tissues, as summarised in Table 1.[43,46,47,49] If these mRNAs are translated into proteins for sex steroid receptors, ocular surface tissues may be target sites for sex hormones and thus may be subject to the local action of these sex hormones. This supports intracrinology where sex hormones produced by intracrine processes exert their action locally within the same cells in which they were formed, before being metabolised locally. [50]

The presence of mRNA alone for AR/ER/PR does not prove that these mRNAs are translated. However, AR, ER and PR protein have been found in the epithelial cell nuclei of human meibomian glands, lacrimal glands, cornea, forniceal and bulbar conjunctiva which implies translation. [39–45] The presence of androgen/ estrogen/ progesterone receptor protein suggests these receptors may facilitate sex hormone influence on the function, structure and pathology of the tear producing tissues. [39] However, no association has been reported between the number of estrogen receptors in meibomian gland basal cells and dry eye symptoms, tear quality (Tear Break Up Time [**TBUT**]), or tear volume (Schirmer score).[42]

3. Gene regulation of ocular surface tissues by sex hormones

To establish whether sex steroids have sex-specific or antagonistic effects, studies have examined the influence of sex hormones on gene expression in epithelial cells of human meibomian glands and conjunctiva, and mice lacrimal glands and meibomian glands. Sex hormone effect on gene expression is not limited to sex hormones produced by intracrine processes, therefore animal studies are used to support the two published human studies discussed in this section.

Sex appears to have a significant influence on gene expression in ocular surface tissues, affecting genes responsible for a broad range of processes. [51–53] However, the influence of sex on gene expression seems to be tissue-specific, with the majority of sex related differences in gene expression in the mouse meibomian gland being different to

those in the mouse lacrimal gland.[54] The most highly expressed genes in human meibomian glands were unique to those in the human conjunctival epithelia, thus gene expression appears to be tissue-specific in humans as well as mice. [52]

Treatment of immortalised human meibomian gland epithelial cells (iHMGEC) and conjunctival epithelial cells (iHCEC) in serum-free medium with DHT was found to influence expression of approximately 3000 genes. [53] in iHCEC DHT enhanced expression of genes involved in the development of the epithelium, wound healing and regeneration, whilst suppressing genes related to immune response and mitotic cell cycle.[53] Androgen treatment in the meibomian gland increased expression of genes for lipogenesis and suppressed genes for keratinisation.[53] Some genes were up/down regulated in both tissues, however as described above, most gene regulation was unique to the meibomian or conjunctival epithelium cells.[53] These findings by Khandelwal et al suggest that androgens appear to be beneficial to the physiology of meibomian glands and conjunctiva.

The only study to look at the effect of both estrogens and androgens *in vitro* in human ocular surface tissues (sex unknown) is by Schroder et al.[52] This study used 1nM 17β-Estradiol and 10nM DHT to modulate gene expression of meibomian gland dysfunction (**MGD**) associated markers in iHMGEC.[41] iHMGEC cultured in serum-free medium were compared to those cultured in serum-containing medium. Both estradiol and DHT increased gene expression for keratinisation in serum-free medium and, interestingly, DHT also down regulated gene expression of lipid synthesis enzymes.[41] These findings contrast with the positive effects of androgens on iHMGEC, found by Khandelwal[58] and in mice studies.[55–57] Schroder et al[52] also found estradiol to increase genes for proliferation of iHMGEC, which disagrees with the findings of a preliminary study which found estradiol to decrease proliferation. [58] The contradictory results could be explained bydifferences in methodology between research groups, including, but not limited to, the use of serum containing medium, hormone concentrations studied and the use of immortalized cell lines. As with topical or systemic treatment *in vivo,* the effect of estrogen on human ocular tissues remains unclear. The effect of sex hormones on gene expression in human ocular tissues is complex. With only two published studies investigating these effects it is not yet possible to form firm conclusions.

Testosterone significantly altered the expression of thousands of genes in the lacrimal and meibomian glands of mice.[57] Interestingly, androgens impacted expression of genes involved in lipid metabolism and inhibition of keratinization in the mouse meibomian gland.[57] This agrees with the study of iHMGEC by Khandelwal et al.[53] Many of the biological and functional effects of androgens were the same in both males and females. [57,59]Estrogen and progesterone also influenced expression of numerous genes in the mouse lacrimal gland [59] and meibomian gland. [60] However, their effects tended to be unique to those of androgens and the number of common genes was limited: estradiol, progesterone or a combination of both sex steroids significantly influenced less than 7% of genes controlled by androgens.[57] Although sex hormones impacted expression of thousands of genes in the lacrimal and meibomian glands of mice, testosterone and estrogen appear to have different effects. Testosterone appears to promote meibomian gland function, which agrees with studies of androgen treatment (see review by Truong et al).[61]

The absence of estrogen, as a result of aromatase elimination, was associated with up/downregulation of thousands of genes in the mouse meibomian gland and lacrimal gland.[62,63] More than 90% of these aromatase-linked genes were sex-specific which could explain the sex related differences of the mouse meibomian and lacrimal glands.[62,63] There was no effect on tear volume in females, however a significant increase in tear volume in male mice was observed[62,63] which suggests sex specific influences. Thus it appears that estrogen and aromatase play an important part in gene regulation in mice meibomian and lacrimal glands. [62,63] However this effect appears to be sex specific.

In summary, there are very few published human studies of the effect sex hormones have on gene regulation in tear producing tissues. Mice studies suggest testosterone and estradiol influence expression of thousands of genes in the lacrimal gland and meibomian glands and that estradiol has contrasting effects to testosterone's stimulatory effect of meibomian glands. To understand the true nature of the role of sex hormones on human ocular surface tissues, further studies are required.

B. Measurement of sex hormone levels at the ocular surface

Many studies examining hormones are performed using animals; whilst these have their logistical benefits, including having larger sample sizes available and ability to control

environmental conditions, the outcomes may not be generalizable to humans. Lower animal species, such as rats and rabbits, are different to humans since they lack intracrine synthesis of hormones. [23] There are many other variables which influence the effects of hormones, including species, sex, age, experimental procedure and strain of hormone used. [64]

One difficulty of investigating local sex hormones in ocular tissues is the requirement for human tissue, which is not feasible in large scale studies. There are studies which use human ocular tissue following surgical removal, but these are rare and small scale.[46] Another option is to measure sex hormones in samples such as tears, meibum or through conjunctival impression cytology, which (although this approach may have other limitations) may be harvested non-surgically.

Sex hormone metabolites may enter the tear-film as a constituent of lacrimal gland secretions or within meibum, as a possible result of the holocrine nature of meibomian glands. [65,66] This speculation is supported by the finding of 17β-Estradiol and progesterone in human tears (using immunoassay kits, in a preliminary study) at concentrations which correlated significantly with serum levels. [67]

Due to low concentrations of hormones and the small sample volumes available from collection of tears, meibum or impression cytology, highly sensitive, reliable and repeatable methods are required. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) is one such method and studies utilizing and developing these methods have begun to be published,[68] setting the standard for future studies. They have the advantage of improved reproducibility, accuracy and sensitivity in comparison to immunoassays.[69–71] To our knowledge, mass spectrometry methods to measure sex hormones in human tears or meibum have not been published to date. A preliminary study managed to successfully detect pregnenolone, progesterone, DHEA, androstenedione and testosterone, as well as corticosteroid metabolites, in human tears using LC-MS/MS.[72] LC-MS and Gas chromatography –MS(GC-MS) methods were developed to detect 9 steroids (testosterone, androstenedione, cortisol, DHEA-S, DHEA, cortisone, β-estradiol, progesterone and androst-5-ene-3,17-diol) and serotonin in human tears, however levels were too low to reach the required sensitivity.[73] New instrumentation, with increased sensitivity, provides the possibility for future successful mass spectrometry measurement of sex hormones in human tears.

C. Sex hormone treatment and dry eye.

Due to the difficulties of measuring local levels of sex hormones, as discussed in section III B, the relationship between intracrine sex hormones and clinical signs or symptoms has not previously been investigated. Truong et al examined evidence for the role of systemic sex hormones in the aetiology of dry eye and the effect of systemic sex hormones on ocular function.[61] They concluded that androgens may have a beneficial impact on the lacrimal glands and meibomian glands, and that androgen deficiency is a contributing factor to dry eye aetiology. They suggested that the impact of estrogen is more uncertain, with studies finding contradictory effects of exogenous estrogen on ocular structures, shown by the unresolved relationship between HRT and dry eye signs and symptoms.[61]

It is important to note that although studies thus far have looked at associations between serum sex hormones and dry eye, serum sex hormones represent only a small portion of sex hormones made in the body, since all estrogens and most androgens are produced and metabolized locally in peripheral tissues post-menopause.[22,26,30] Topical treatment with sex hormones may be a viable route of administration for local treatment of dry eye. Studies have used two different routes of topical administration: cream applied to the eyelids or eye drops, summarized in Table 2.

Table 2: Summary of the effects of topical androgens and estrogens on symptoms and signs of dry eye.
Topical treatment was in the form of eye drops or cream applied to the eyelids.

Author	N° subjects	Treatment	Dosage	Duration	Placebo	Effect on Dry eye
Androgens						
WO Patent No 1994004155 A1, 1994 [74]	F=1	1.5% DHEA drops	/	3 weeks	N	Improved symptoms, But and lipid layer, reduced Schirmer's
Worda et al 2001* [75]	M=1	3% Testos cream	t.i.d.	3 months	N	Improved symptoms and lipid layer, restored tear film
Connor, et al 2001 [Conference][76]	F=1, M=9	1% DHEA drops	q.i.d.	2 weeks	Y	Improved symptoms, Schirmer's and TBUT
Connor 2002 [Conference] [77]	F=15, M=5	2.5% Testos cream	b.i.d.	3 weeks	Y	Improved symptoms and Schirmer's
Connor , et al 2002[Conference] [78]	F=9, M=11	1% Testos, 1% DHEA drops	q.i.d.	2 weeks	Y	DHEA improved BUT & Schirmer's Testos decreased Schirmer's
US Patent No 6659985 B2, 2003[79]	F=4, M=1	2.5% DHEA cream	t.i.d.	2 weeks	N	Improved symptoms & CL WT. Improved Schirmer's and TBUT in male subject only
	F=2	2.5% Testos cream	b.i.d.	2 weeks	N	Improved symptoms , CL WT, Schirmer's and BUT
Connor 2003 [Conference][80]	F=25, M=3	3% Testos cream	b.i.d.	2 weeks	Y	Improved symptoms and Schirmer's
Schiffman 2006[Conference] [81]	179	0.01%, 0.03%, 0.1% Testos	/	6 months	Y	Improved MG secretions
Connor 2007[Conference] [82]	F=40, M=10	5% Testos, 20% Prog cream	b.i.d.	/	N	Improved symptoms and TBUT
Connor 2012[Conference][83]	F=30	5% Testos cream	b.i.d.	1 month	N	Improved TBUT
Estrogens						
Lubkin 1992[Conference][84]	F=14	E2 drops. unknown%	q.i.d.	2 months	Other eye	Improved symptoms
US Patent No 6096733, 1998 [85]	F=45	0.1%, 0.25% E2 drops	q.i.d.	90 days	Y	Improved symptoms and TBUT
	F	0.05% E2 drops	t.i.d.	10 days	Other eye	Maturation conjunctival epithelium
Akramanian et al * 1997 & 1998 [86,87]	F=20	3% E2 ointment	/	1 week	Y	Improved symptoms, Schirmer's and TBUT
Sator et al* 1998[88]	F=84	0.025% E2 drops	q.i.d.	4 months	Y	Improved symptoms and Schirmer's

Abbreviations: *indicates published studies, [Conference]: conference proceedings, F: Female, M: Male, Testos : testosterone, Prog: progesterone, E2: Estradiol, /: unknown, b.i.d: twice daily, t.i.d. three times daily, q.i.d. four times daily, Y: yes, N: no, CL WT: contact lens wearing time, TBUT; tear break up time, MG: meibomian gland.

In summary, eye drops and cream applied to the eyelids, supplemented with testosterone or DHEA have a beneficial effect on symptoms, tear stability and quantity.[75,77,80,83],[74,78] However, evidence is weak with an absence of placebo controlled published studies which are required to improve our understanding of the possible application of topical androgens for clinical treatment of dry eye.

There are a very limited number of studies researching the effect of estradiol therapy on dry eye, including only two published controlled studies, one of which has many possible confounding factors.[88] Evidence seems to suggest that topical estradiol may be beneficial for the treatment of dry eye. This is in agreement with studies which found systemic estrogen treatment to improve dry eye,[87–93] but disagrees with studies which found systemic estrogen therapy to exacerbate dry eye.[94–96] Although systemic estrogen therapy has conflicting results, the limited results from studies of topical estradiol therapy (including preliminary studies) suggest a positive effect of topical treatment, which may be more associated with local synthesis of sex hormones.[24]

Topical estrogen and androgen therapy appears to have a positive effect on signs and symptoms of dry eye; however published evidence is weak with the majority of studies being preliminary.

IV. SUMMARY

This review aimed to provide a comprehensive discussion focussed on the impact of local sex hormone synthesis on dry eye. It was necessary to discuss the wider topic of intracrinology before focussing on ocular structures. Despite the increase in knowledge of local hormone synthesis, much of the recent literature focusses on the effects of circulating, rather than local, sex hormones on signs or symptoms of dry eye. This is likely due to the relative ease of systemic measurement in comparison to local.

Much research regarding sex hormones and the ocular surface has been performed on rats and mice to show the presence of sex hormone mRNA in, ocular structures; the meibomian and lacrimal glands have been of particular interest in published research to date, likely due to their role in tear formation. Labrie describes intracrinology as “the formation of active hormones that exert their action in the same cells where synthesis

took place without release into the pericellular compartment."[22,23] The presence of steroidogenic enzymes in the ocular surface tissues suggests that human ocular surface tissues are capable of local formation as well as inactivation of sex steroids. The presence of AR, ER and/or PR mRNA and protein suggests that these ocular tissues are target sites for sex hormones and that once synthesized, the locally produced sex steroids may exert their action within the same tissues. This is supported by human and mice studies which have demonstrated that androgens and estrogens regulate numerous genes in the meibomian gland, lacrimal gland and conjunctiva.

The important physiological role of estrogens and androgens in the function and structure of ocular tissues calls for an improved understanding of intracrine hormone synthesis and their metabolism. Measurement of local sex hormone levels has its challenges, including the procurement of human ocular surface tissue. Measuring levels of sex hormones and metabolites in tears or meibum thus provides an estimateof ocular surface tissue levels, without the need for tissue excision. Technological advances, including in LC-MS and GC-MS, will provide the sensitivity required to measure the low levels of sex hormones and their metabolites which are present in human tears and meibum.

Developments in technology not only allow our understanding of how sex hormones influence the ultrastructure of ocular tissues, but also uncover the presence of the biological machinery needed for intracrine hormone synthesis. Thus it is anticipated that with further technological developments, rapid progress will be made in understanding how sex hormones affect ocular tissues and contribute to the development of dry eye. Clarification of the action of sex hormones on ocular surface tissues and their contribution to dry eye is essential for the development of suitable hormone based treatments for dry eye.

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FIG 1: Schematic representation of human endocrine (adrenal) and intracrine steroidogenic pathways. Endocrine processes allow cholesterol to be converted into progestagens then androgens, which are then transformed into estrogens. DHEA and DHEAS are secreted by the adrenals and are used for steroidogenesis in peripheral intracrine tissues. Corticosteroids are also included as they are formed from the same precursors (progesterone and 17α-hydroxyprogesterone). Corticosteroids include mineralocorticoids (primary being aldosterone) and glucocorticoids (primary being cortisol).*Italic* boxes represent enzymes involved.

Adapted from Labrie 2007.[28]

Abbreviations: DHEA: dehydroepiandrosterone, DHEAS: dehydroepiandrosterone-sulphate, DHT: dihydrotestosterone

FIG 2: Schematic representation of ovarian and adrenal sources of sex steroids in premenopausal women. Humans have adrenal glands which secrete large quantities of the precursor DHEA which is converted into progestogens, androgens and estrogens in peripheral tissues. After menopause ovarian estradiol secretion ceases, thus 100% of sex steroids are then made locally in tissues by intracrine pathways. LH stimulates the secretion of sex hormones from the gonads and ACTH modulates adrenal secretion of DHEA.

Adapted from Labrie 2003. [30]

Abbreviations: LH: Luteinising hormone, ACTH: adrenocorticotropin, DHEA: dehydroepiandrosterone.

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