Sex Hormones, Ocular Surface Sensitivity and Dry Eye Symptoms and Signs in Postmenopausal Women

Author:
Badarudin, Noor

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Sex Hormones, Ocular Surface Sensitivity and Dry Eye Symptoms and Signs in Postmenopausal Women

Noor Ezailina Badarudin

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Sex hormones may influence symptoms and signs of dry eye. This thesis aims to identify relationships between circulating sex hormones and dry eye symptoms and signs and to investigate the effects of transdermal sex hormones treatment on these variables.
Method development was undertaken for ocular surface sensitivity and the Cochet-Bonnet aesthesiometer was selected above a pneumatic instrument based on validation and repeatability data.

A cross-sectional study of 76 normal-to-mild dry eye subjects demonstrated inverse relationships between circulating androgens, their precursors or metabolites and symptoms ($r = -0.34$, $p = 0.003$) and tear osmolarity ($r = -0.30$,$p = 0.03$) and positive associations with corneal sensitivity ($r = 0.28$, $p = 0.02$) and tear volume ($r = 0.35$, $p = 0.002$). In contrast, oestradiol was positively associated with symptoms ($r = 0.31$, $p = 0.03$) in women only. However, when potential confounding was considered, neither androgen nor oestradiol were able to predict symptoms in regression analysis.

A similar analysis in 45 postmenopausal women with dry eye, showed that oestradiol was associated with corneal staining ($r = 0.56$, $p = 0.001$), but there were no relationships between symptoms and hormone levels in either univariate or multivariable analysis.

A double-masked randomised placebo-controlled 8 week pilot intervention study was conducted on 40 postmenopausal women with dry eye using transdermal testosterone, oestradiol or their combination. Key findings included a significant improvement in ocular symptoms in the testosterone, combination and placebo groups ($p < 0.1$) and increased in tear volume in the combination group ($p < 0.05$).

While dry eye signs and symptoms in a mild dry eye population show associations with hormones, specifically an improvement with androgen and the reverse with oestradiol, these relationships were not evident in multivariable analyses. Corneal staining was associated with oestradiol in postmenopausal women with dry eye and an intervention study suggested that a combination of testosterone and oestradiol improved both the signs and symptoms of dry eye in this group.

This thesis describes a series of studies to establish the influence of hormones on dry eye signs and symptoms in different populations. Such relationships are likely to vary with hormone levels, the combination of key hormones and dry eye status.

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ORIGINALITY STATEMENT

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Signed .....

Date 04/08/2014

PUBLICATIONS AND PRESENTATIONS

Poster: Repeatability of the Belmonte Ocular Pain Meter and Comparison to the Cochet-Bonnet Aesthesiometer

Badarudin N, Golebiowski B, Stapleton F

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Cecilia Chao, Fiona Stapleton, Ezailina Badarudin and Blanka Golebiowski

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ABSTRACT

Sex hormones may influence symptoms and signs of dry eye. This thesis aims to identify relationships between circulating sex hormones and dry eye symptoms and signs and to investigate the effects of transdermal sex hormones treatment on these variables.

Method development was undertaken for ocular surface sensitivity and the Cochet-Bonnet aesthesiometer was selected above a pneumatic instrument based on validation and repeatability data.

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oestradiol, these relationships were not evident in multivariable analyses. Corneal staining was associated with oestradiol in postmenopausal women with dry eye and an intervention study suggested that a combination of testosterone and oestradiol improved both the signs and symptoms of dry eye in this group.

This thesis describes a series of studies to establish the influence of hormones on dry eye signs and symptoms in males, menstruating and postmenopausal women. Such relationships are likely to vary with hormone levels, the combination of key hormones and dry eye status. The inferior conjunctival sensitivity was among the significant predictors of symptoms, revealing the importance of ocular surface sensitivity as an important dry eye clinical indicator. The corneal sensitivity measurement may be affected by the androgen level especially in males.

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This thesis is dedicated to my dear father

Addendum to statement of originality

There were several people and organisations involved in the work presented in this thesis.

Chapter 2

The measurement of Cochet-Bonnet filament diameter thread was conducted by Dr Klaus Ehrmann. The calibration of the Cochet-Bonnet 0.12 filament was performed by Cecilia Chao (Section 2.4). The verification of the Belmonte Ocular Pain Meter was conducted by Dr Edward Lum.

Chapter 3

All clinic record forms (CRF) used in the clinical study was prepared for online submission using Key Survey (WorldApp, US) by Moneisha Gokhale. The screening and recruitment of the participants, dispensing symptoms of ocular discomfort online questionnaires were conducted together by Noor Badarudin and Moneisha Gokhale. The evaluation of the clinical indicators which included tear function and ocular surface integrity was conducted by Moneisha Gokhale. Sex hormone concentration measurements were conducted by Dr Ulrike Hampel and Noor Badarudin at the School of Optometry and Vision Science (SOVS).

Chapters 4 and 5

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IBM SPSS Version 21 (Statistical Package for The Social Sciences, Chicago, Illinois) was used in data analysis throughout the studies described in this thesis.

Statistical analysis consultation was provided by Dr Thomas Naduvilath

Writing assistance was provided by the tutors at the Learning Centre UNSW and Dr Judith Flanagan

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SYMBOLS AND ABBREVIATIONS

%	percentage
β	beta
°C	degrees centigrade
≤	less than or equal to
<	less than
=	equal
>	more than
n	number of participants
x	times
mm^2	millimetre square
µL	micro litre
mm	milimetre
ml/mL	millilitre
mOsmo/L	milli osmol per litre
A.M	morning
P.M	afternoon
p	level of significance
r	correlation coefficient
ADT-G	Androsteroneglucuronide
ATP	Adenosine triphosphate
BOPM	Belmonte Ocular Pain Meter
cAMP	cyclic adenosine monophosphate
COBO	Cochet-Bonnet
CoR	Coefficient of repeatability
DEQ	Dry Eye Questionnaire
DNA	Deoxyribonucleic acid
DHEA	Dehydroepiandrosterone
DHEA-S	Dehydroepiandrosterone Sulfate
DHT	Dihydrotestosterone
ELISA	enzyme-linked immunosorbent assay
HRT	Hormone Replacement Therapy

LoA	Limits of agreement
mRNAs	Messenger ribonucleic acid
MENQOL	Menopause-Specific quality of life
NRS	Numerical Ratings Questionnaire
NIBUT	non-invasive break up time
OCI	ocular comfort index
OSDI	ocular surface disease index
PRT	phenol red thread
SD	standard deviation
SHBG	sex hormone binding globulin
3α-diol G	5alpha-androstane-3alpha and 17beta-diolglucuronide

CHAPTER 1

Literature Review

1.1 Introduction

Dry eye is defined as “a disorder of the ocular surface that results in symptoms of discomfort, visual disturbance and tear film instability with increased osmolarity of the tear film and inflammation of the ocular surface, which may lead to ocular surface damage” [Dry Eye Workshop report (DEWS)] (Lemp et al 2007). The ocular surface plays a role in preventing eye injury, maintaining a smooth refractive surface for vision and protecting the eye against adverse physical and environmental conditions (Pflugfelder & Stern 2004, Stern et al 1998) and this role is modulated by a properly functioning tear film (McKown et al 2009). Consequently, a compromised lacrimal functional unit might result in dry eye.

Dry eye disease is among the most frequently reported eye problems, with an estimated prevalence of 7% to 34% across the globe (Doughty et al 1997, Lee et al 2002, Lin et al 2003, McCarty et al 1998, Moss et al 2000, Shimmura et al 1999). Rates vary widely because of different diagnostic criteria used in reported studies. Dry eye symptoms affect quality of life by reducing productivity at work, impairing computer use, reading and driving (Miljanovic et al 2007, Patel et al 2011). Generally, reduced work productivity leads to a rise in health care expenditure, that may be several times more than the cost of lost productivity (Mattke et al 2007). The total annual healthcare cost of one thousand dry eye syndrome sufferers managed by ophthalmologists ranged from US$0.27 million in France to US$1.10 million in the UK, and the real cost might possibly be higher since many dry eye sufferers opt instead for over-the-counter artificial tears and other medications (Clegg et al 2006) and utilise optometry and general practitioner care.

Several large-scale epidemiological studies have lent support to the idea that dry eye is most prevalent in elderly women (Chia et al 2003, Lin et al 2003, McCarty et al 1998, Moss et al 2000), suggesting that age and gender are the key contributing factors to dry eye. In addition, the compromised levels of the ovary produced hormones in this

population (Overlie et al 1999) might suggest their involvement in dry eye. Therefore, an increased understanding of how age, gender and sex hormone levels contribute to dry eye might help in the amelioration of this condition.

Postmenopausal women are defined as those who have experienced the permanent cessation of a menstrual cycle for 12 months, due to the loss of ovarian follicular activity (Utian 2004). The median age for menopause is 51 years and this may vary by race, social status, smoking habits, history of heart disease and prior use of oral contraceptives (Gold et al 2001).

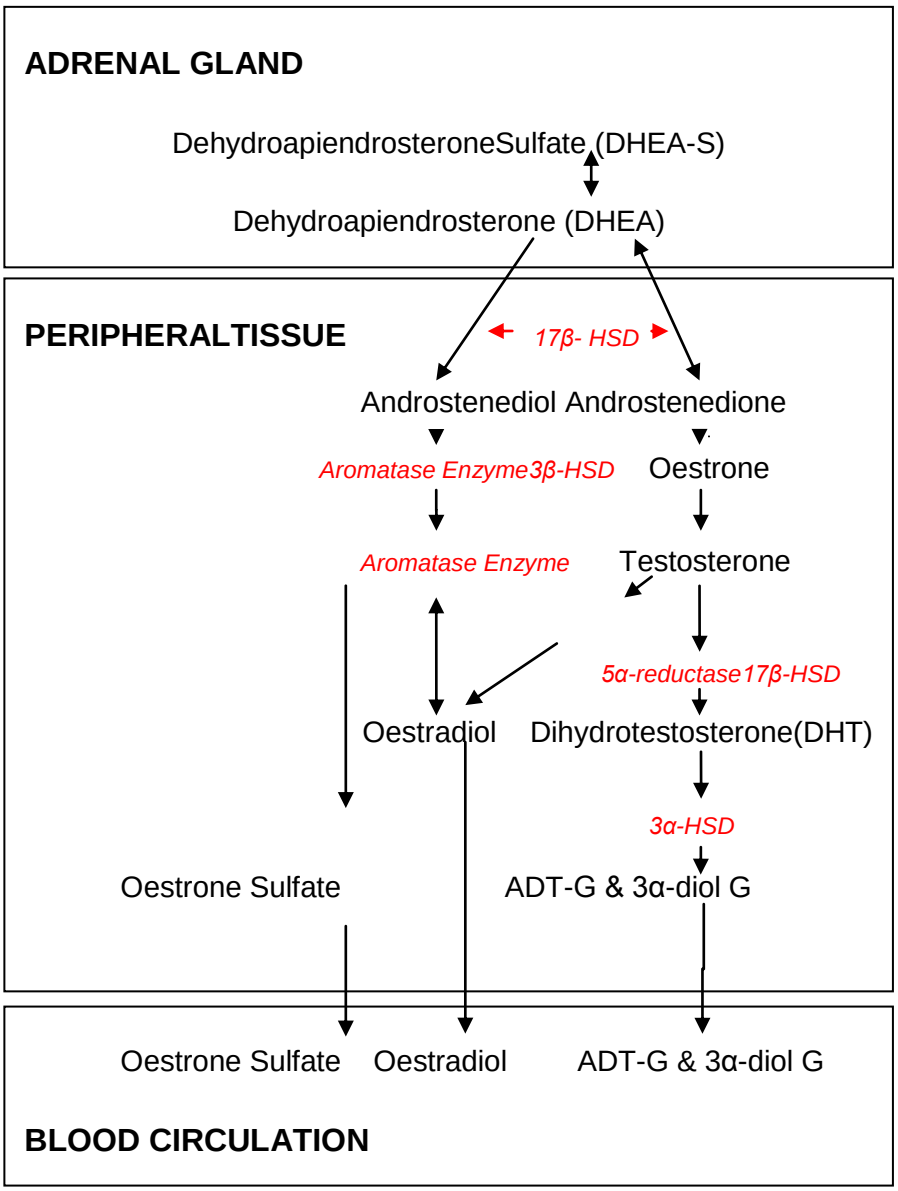
There is good evidence that meibomian and lacrimal glands are regulated by ovary-produced sex hormones such as androgen, oestrogen and progesterone (Khandelwal et al 2012, Krenzer et al 2000, Schirra et al 2005, Sullivan et al 2002c, Sullivan et al 2000). This theory is supported by the identification of androgen, oestrogen and progesterone receptor messenger ribonucleic acid (mRNAs) (Wickham et al 2000) and steroidogenic enzyme mRNAs (Schirra et al 2006) in meibomian and lacrimal glands. Changes in these hormone levels might be associated with reduced tear production (McCarty et al 1998) and increased tear evaporation around the sixth decade of life in postmenopausal women (Guillon & Maïssa 2010).

Ocular surface sensitivity is an important clinical indicator for corneal health. Reduced corneal sensitivity could be harmful to the long-term health of the cornea as the eye relies on the corneal nerves to detect foreign bodies that could damage its most anterior layer. The sex hormones receptor mRNAs were also identified on the palpebral and bulbar conjunctiva and cornea (Wickham et al 2000). In this thesis, sex hormones are speculated to directly affect ocular surface sensitivity through the hormone-receptor activation (Bereiter et al 2005, Brown et al 1996, Romano et al 1988) or indirectly through a neural feedback loop, linking the lacrimal gland and ocular surface (Mathers 2000, Stapleton et al 2013). Therefore, changes to physiological levels of androgen, oestrogen and progesterone may affect ocular surface sensitivity.

The synthesis of sex hormones can occur in the peripheral target tissue, in a process termed intracrinology (Labrie et al 2003) (Figure 1.1). Intracrinology occurs in the ocular tissue where the adrenal sex steroid precursor, Dehydroepiandrosterone (DHEA), is converted into testosterone and oestradiol. The testosterone is eventually

transformed into androgen metabolites including androsteroneglucuronide (ADT-G); and 5alpha-androstane-3alpha and 17beta-diolglucuronide (3α-diolG) which enter the circulation. The intracrine process is an important alternative source of androgen (40% in men, 75% in premenopausal women and almost 100% in postmenopausal women) and oestrogen production (almost 100% in postmenopausal women) (Labrie 1991, Labrie 2010). However, based on the literature referred to above, the hormones produced are likely to be insufficient to maintain the ocular surface hydration, sensitivity and homeostasis in postmenopausal women, compared to premenopausal women with functioning ovaries. Figure 1.1 illustrates intracrinology and is adapted from Labrie et al. Molecular and Cellular Endocrinology 1991, Labrie et al. J Endocrinol 2005, Van Luu-The and Labrie Progress in Brain Research 2010 and Truong et al. Clin. Exp. Optom 2013.

Figure 1.1 The Intracrinology Process



NB: Enzymes required for the interactions are printed in red
ADT-G: androsteroneglucuronide
3α-diol G: 5 alpha-androstane-3 alpha and 17 beta-diolglucuronide

As changes to physiological levels of oestrogen, androgen and progesterone might affect ocular symptoms and signs (Forsblad-d'Elia et al 2009, Gagliano et al 2014, Krenzer et al 2000, Marcozzi et al 2003, Mathers et al 1998, Millodot & Lamont 1974, Riss et al 1982, Sahin & Kartal 2011, Scuderi et al 2012, Sullivan et al 2003, Versura et al 2007),hormone replacement therapy (HRT), which is an administration of one or more female hormones, (commonly oestrogen alone or with progesterone), has been investigated as a potential remedy for dry eye (Adatia et al 2004, Affinito et al 2003, Akramian et al 1998, Coksuer et al 2011, Guaschino et al 2003, Jung et al 2010, Sator et al 1998, Taner et al 2004). The main indication for HRT use in postmenopausal women is the relief of systemic menopause symptoms such as hot flushes and night sweats (Avis et al 2001). The prevalence of HRT use between March 2009 and March 2010 was 3%in Australia(Morgan 2011); 8.7% between January 2001 and January 2004 in The Netherlands (de Jong-van den Berg et al 2006) and 12% between January and May 2004 in the United States (Ness & Aronow 2006).

The effects of HRT on dry eye signs and symptoms are unclear because of differences in the methodology such as the duration of therapy (Uncu et al 2006), number of years since menopause (Erdem et al 2007),washout period of previous HRT usage (Altintaş et al 2004), lack of placebo control in a majority of studies and various hormone administration methods.

In menopause, changes in oestrogen, androgen and progesterone levels take place after cessation of ovarian activity. Since dry eye is mostly reported in postmenopausal women (Chia et al 2003, Lin et al 2003, McCarty et al 1998, Moss et al 2008), it is therefore hypothesised that ocular symptoms are also caused by the changes in these sex hormone levels. The types of ocular symptoms reported by postmenopausal women are listed in Table 1.1.

Table 1.1 Frequency based Report on Ophthalmic Complaints in Menopause (Metka et al 1991)

Deterioration in visual acuity
Feeling of dryness
Smarting
Pressure sensation
Sensitivity to light
Flickering
Blurring of vision
Increased lacrimation
Tired eyes
Swollen eye lids
Scratching sensation
Gummed up eyes
Reddened eye lids
Coordination problems
Foreign body sensation
Sensation of coldness
Transient visual disturbance
Sensation of contraction
Sunken eyes

Apart from symptoms, other dry eye clinical signs such as ocular surface sensitivity (Millodot & Lamont 1974, Riss et al 1982) and tear function are also affected by sex hormone levels regardless of menopausal status, both in women with Sjögren's syndrome (Forsblad-d'Elia et al 2009, Sullivan et al 2003) and those without (Gagliano et al 2014, Mathers et al 1998, Versura et al 2007). In addition, ocular surface sensitivity was also affected by sex hormone levels (Millodot & Lamont 1974, Riss et al 1982), although it is not generally considered to be a dry eye clinical sign. Reduced corneal sensitivity is associated with other entities such as diabetes (Cousen et al 2007), neurodegenerative diseases (Alzheimer, Multiple sclerosis, Parkinson's disease, Friedreich's ataxia and epilepsy) (Örnek et al 2014), and herpes simplex (Liesegang et al 2008).

Only a few studies have presented direct associations between circulating sex hormone levels and dry eye symptoms, and signs such as tear function in women with Sjögren's syndrome (Taiym et al 2004) and without (Gagliano et al 2014, Mathers et al 1998, Scuderi et al 2012). Furthermore, no direct association has been reported between sex hormones (androgen, oestrogen and progesterone) levels and ocular symptoms or dry eye signs in a normal population of either gender. Examination of these associations in a normal population is important to understand the impact of

age and gender on the relationship between dry eye and sex hormones. Although progesterone receptor mRNA is located on the ocular surface (Wickham et al 2000), and progesterone is proposed to have nociceptive effect at central nervous system (CNS) which may affect ocular surface sensitivity (Kuba et al 2006, Romano et al 1988), there is no published evidence of an association between progesterone level and dry eye. This thesis attempts to clarify the relationship between sex hormones and dry eye symptoms, signs and ocular surface sensitivity. To accomplish this, methods were developed to evaluate ocular surface sensitivity, which may be affected by dry eye. A normal population of males and females establishes the links between sex hormones and ocular sensitivity, and symptoms and signs of dry eye were initially evaluated in a normal population to identify associations and understand the impact of age and gender on these variables. A further investigation of the effects of sex hormone treatments on dry eye was conducted on postmenopausal women to elucidate the mechanism of any identified associations and to identify a potential remedy for dry eye.

1.2 Sex Hormones and Dry Eye

1.2.1 Effects of Sex Hormones on Ocular Surface Sensitivity

Dry eye patients often report symptoms of burning, stinging, dryness or discomfort (Doughty et al 1997, Lee et al 2002, Lin et al 2003, McCarty et al 1998, Moss et al 2000, Shimmura et al 1999). Dry eye symptoms might originate from either direct or indirect stimulation of the ocular surface sensory receptors (Belmonte et al 2004, Belmonte et al 1997, Johnson 2009, Julius & Basbaum 2001, Luo et al 2004). The possible link between the sensory function of the ocular surface and symptoms highlights the potential importance of ocular surface sensitivity in preserving ocular surface health (Barboza et al 2008, Benitez-del-Castillo et al 2001, Bourcier et al 2005, De Paiva & Pflugfelder 2004, Situ et al 2008b, Stapleton et al 2013, Toker & Asfuroglu 2010, Tuisku et al 2008, Versura et al 2006, Xu et al 1996). Ocular surface sensitivity is an important clinical indicator for corneal health. For instance, reduced corneal sensitivity could be harmful to the long-term health of the cornea as the eye relies on the corneal nerves to detect foreign bodies that could damage its most anterior layer. This conscious sensation can be elicited by mechanical, chemical or thermal

stimulation, which is delivered using different stimulus types. Mechanical stimuli activate mechano-nociceptors and polymodalnociceptors on the ocular surface, which are most likely responsible for producing sharp pain (Belmonte et al 2004, Belmonte et al 1997).

Nociceptors are the neurons possessing thin myelinated (Aδ) or unmyelinated (C) axons which terminate peripherally (Belmonte et al 2004, Bron et al 1997,Stapleton et al 2013). Upon stimulation, sensory nerves in the ocular surface transmit impulses to the ophthalmic branch of the trigeminal nerve which synapses within the central nervous system (CNS), when a suitable threshold is reached (Dastjerdi & Dana 2009, Stapleton et al 2013). Oestrogens and androgens are also synthesised in the CNS and may influence pain or sensory transmission through the CNS, which is responsible for modulating the pain signal nociceptors based on animal studies (Beyenburg et al 2001, Lephart 1996, Shibuya et al 2003). There are two types of oestrogen receptors, (ERα) and (ERβ), which are located on neurons of the trigeminal brainstem complex (trigeminal subnucleuscaudalis) and as stated above are hypothesised to modulate pain perception (Bereiter et al 2005). The oestradiol-receptor activity rapidly attenuates ATP - induced calcium signalling with the expressibility of ERα at dorsalroot ganglion cells (Chabin & Micevych 2005) which likely results in anti-nociceptive effects (Cao et al 2012, Ma et al 2011). In contrast, oestrogen can potentially increase enkephalin gene expressibility in the spinal cord which leads to nociceptive effects (Allen & McCarson 2005, Amandusson et al 1999). Progesterone might also be involved in the nociceptive effect by prolonging the elevation in preproenkephalin mRNA levels at ventromedial hypothalamus of rats as demonstrated after oestrogen administration (Romano et al 1988). However, progesterone showed the reverse effects of oestradiol in formalin-induced nociception of female rats (Kuba et al 2006). Androgens are able to antagonise oestrogen receptor responses which could result in an increase in pain sensitivity(Brown et al 1996). In addition, testosterone can both increase (Forman et al 1989, Frye & Seliga 2001) and decrease (Nayebi & Ahmadiani 1999) the painful stimuli sensitivity. Hormonal-related pain sensitivity at the CNS translated to the ocular surface, might lead to changes in ocular surface sensitivity during phases of the menstrual cycle, and as affected by age and gender.

As early as in 1933, Herren et al suggested that pain sensitivity may be related to sex

hormone levels with an increase in the two point tactile sensitivity in pre-menstruating as compared to post-menstruating women aged 24-34 years (Herren 1933).

In contrast, a decrease in corneal sensitivity was demonstrated during the oestrogen peak pre-ovulatory phase of the menstrual cycle and pregnancy (Millodot 1977b, Millodot & Lamont 1974, Riss et al 1982). The corneal sensitivity reduction might be caused by corneal oedema (Millodot & Lamont 1974)which occurred with the presence of oestrogen (Spoerl et al 2007).

The relationship between sex hormones and pain sensitivity could be explained by the feedback mechanism of the hormones themselves. The female menstrual cycle is controlled by follicle stimulating hormone (FSH) secreted by the pituitary gland and luteinizing hormone (LH) (Welt et al 2003). The increase in LH and FSH immediately before ovulation induces a gradual increase in oestrogen and progesterone. In female rats, LH surges at the beginning of the luteal phase. This induces the desensitisation of brain opioid receptors, resulting in increased pain sensitivity (Bereiter & Barker 1980, Bereiter et al 2000, Fillingim & Edwards 2001). Furthermore, oestrogen treatments induce receptive field enlargement in mechano-receptive trigeminal neurons in ovariectomised female rats injected with oestradiol in the face, which suggests an expansion of the region of sensitisation (Bereiter & Barker 1980). If this is applicable to humans, it may explain the increase in dry eye symptoms in HRT users among postmenopausal women (Schaumberg et al 2001). However, if the proposed anti-nociceptive theory of oestrogen described in the second paragraph of this section earlier prevails, a reduction in pain sensitivity may reduce symptoms reported by these women. Given these contradictory expectations for the role of oestrogen in modulating somatic sensitivity, it would be relevant to identify the impact of changes in sex hormone levels on ocular sensitivity and symptom reporting.

1.2.1.1 Effects of Age and Gender on Ocular Surface Sensitivity

Gender and age-related differences affect ocular surface sensitivity. Corneal sensitivity measured using a non-contact (Acosta et al 2006, Bourcier et al 2005) and Cochet-Bonnet aesthesiometer reduces with age (Millodot 1977a). However, although corneal and conjunctival sensitivity increase with age, especially in females (Golebiowski et al 2008), there was no difference reported in conjunctival and corneal thresholds between

age groups (Situ et al 2008b) where both studies were using the CRCERT-Belmonte non-contact aesthesiometer (CBA). Interestingly, the difference in sensitivity between cornea and conjunctiva may become more pronounced with aging (Roszkowska et al 2004), although the statistical analysis of this finding is questionable. With regards to gender, one study demonstrated a higher corneal sensitivity (Acosta et al 2006) with the original Belmonte instrument in females while two studies demonstrated a higher conjunctival sensitivity in females when measured with CBA (Golebiowski et al 2008, Situ et al 2008b). These findings suggest that differences between studies may result from instrumentation and subjects demographics.

1.2.2 **Effects of Sex Hormones on Lacrimal Functional Unit**

The identification of androgen, oestrogen and progesterone receptors (Wickham et al 2000) and their steroidogenic enzyme mRNAs (Schirra et al 2006) in various human ocular tissues including the cornea, bulbar conjunctiva and importantly lacrimal and meibomian glands is considered a milestone in the understanding of the relationship between sex hormones and dry eye. Intracrinology could explain the translation of steroidogenic mRNAs intracellularly (Labrie et al 2003) at ocular sites and almost 100% of the androgens in postmenopausal women are supplied by this process (Labrie et al 1995, Labrie et al 2003). Once synthesised, free androgen combines with its receptor, and activates the hormone-receptor complex by associating with the appropriate enhancer element, such as the NH_2terminal (Simental et al 1991)in a classical genomic hormone-receptor activation. In non-genomic hormone-receptor activation, the hormone can act through membrane associated specific protein hormones without entering the receptor cells (Gupta et al 2005). In either case, the hormone-receptor complex interacts with a specific DNA sequence within the target cell nucleus and modulates gene transcription and expressibility (Schirra et al 2005, Steagall et al 2002) and promotes mRNA translation (Simental et al 1991, Tsai et al 1998). In the lacrimal and meibomian glands, this activation process of androgen-receptor complex takes place in the acinar epithelial cells (Rocha et al 2000, Sullivan 2004b). Subsequently, androgen regulates the expressibility of thousands of genes in the lacrimal and meibomian glands in mice (Sullivan et al 2009) and in the human meibomian gland and conjunctival epithelial cells (Khandelwal et al 2012), and has an anti-inflammatory

effect in the lacrimal gland (Sullivan et al 1990), as demonstrated in ovariectomised mice. In the human meibomian gland, such gene expressibility by androgen increases numerous processes such as protein metabolism, tissue development, oxido-reductase and peptidase activities, while in the conjunctival epithelial cells, genes related to epithelium development, regeneration, wound healing, and cell migration, among others, are expressed (Khandelwal et al 2012).

In humans, serum testosterone levels are positively associated with tear function in postmenopausal women (Mathers et al 1998) and low testosterone levels are associated with dry eye in women (Mamalis et al 1996). Androgen deficiency has been associated with meibomian gland dysfunction (Cermak et al 2003, Krenzer et al 2000, Sullivan et al 2002c). The presence of androgen receptor protein in acinar epithelial cell nuclei of the meibomian gland may affect the meibomian lipid composition since acinar cells produce proteins that augment both the synthesis and secretion of lipids in response to androgen (Sullivan et al 2000). Furthermore, topical and systemic androgen therapy improves signs and symptoms of dry eye in patients with autoimmune diseases (Bizzarro et al 1987) and non-autoimmune disease (Connor 2003, Nanavaty et al 2013).

Oestrogen appears to antagonise the positive effects of androgen on the lacrimal and meibomian gland secretion and available evidence suggests that endogenous oestrogen has either no impact (Sullivan 2004b) or a negative impact on tear function in postmenopausal women (Mathers et al 1998). Furthermore, treatment with oral contraceptives (Chen et al 2013) and postmenopausal hormone replacement therapy (Schaumberg et al 2001) containing oestrogen have been implicated in increased ocular discomfort and dry eye. In contrast, an improvement in dry eye has been reported in several investigations of oestrogen-alone therapy in postmenopausal women (Sator et al 1998, Scuderi et al 2012).

Activation of the androgen-receptor complex up-regulates lipid production by the meibomian gland (Sullivan et al 2000), which promotes tear stability and prevents tear evaporation (Foulks & Bron 2003). Oestrogen may inhibit lipid synthesis in large sebaceous glands (Thody & Shuster 1989)and as one of sebaceous glands, it is conceivable that the meibomian gland is similarly affected by oestrogen. Furthermore, oestrogen suppresses the genes involved in lipid biosynthesis, mobilisation,

processing, and membrane trafficking (Suzuki et al 2008) in the mouse meibomian gland. In humans, however, one study found that there were no associations between meibomian gland oestrogen receptor expressibility and subjective dry eye symptoms or tear functions (Auw-Haedrich & Feltgen 2003).

Exogenous oestrogen decreases the size of the sebaceous gland of the hamster ear, resulting in shrinkage of the contra-lateral ear gland (Schäfer & Krause 1985). However these investigators suggested that parallel administration of androgens can overcome this effect through competitive inhibition of oestrogen binding to the oestrogen receptor (Jordan et al 1977). This inhibition was dose dependent and that combination therapy may improve gland function. To date only one retrospective study reported the positive effects of the combined treatment on dry eye (Scott et al 2005).

At the corneal surface, oestrogen is believed to play a role in promoting gene expressibility of inflammatory cytokines and matrix metalloproteinases (MMPs)(Suzuki & Sullivan 2005). The release of these pro-inflammatory mediators may contribute to ocular surface inflammation (Yeh et al 2004) and discomfort. However, a more recent study suggested that oestradiol may protect against hyperosmolarity-induced ocular surface inflammation in dry eye (Wang et al 2012). On a more positive note, lacrimal fluid peroxidise, which is an antioxidant and antimicrobial enzyme involved in the protection of the ocular surface, may be up regulated by oestrogen (Marcozzi et al 2003). More investigations are warranted to provide a better understanding of the role of oestrogen on the ocular surface.

1.2.2.1 Effects of Age and Gender on Lacrimal Functional Unit

Aging reduces tear osmolarity (Mathers et al 1996), tear volume (Lamberts et al 1979, Paschides et al 1991, Sakamoto et al 1993, Versura et al 2006) and tear break-up time (Cho & Yap 1993, Patel & Farrell 1989). A reduction in the thickness and area of lacrimal gland and tear evaporation rate (Guillon & Maïssa 2010) were shown in females. Ocular surface staining was, however, not associated with age and gender in a large dry eye epidemiological study (McCarty et al 1998).

Aging is also a major risk factor for meibomian gland dysfunction, based on the human lid margin or meibomian glands (Den et al 2006) signs such as vascularity, cutaneous

hyperkeratinisation and meibomian gland orifice narrowing (Hykin & Bron 1992); telangiectasia, keratinisation, irregular posterior margins, opaque secretions, and changes in the lipid profile of meibomian gland secretions (Ding & Sullivan 2012); a decrease in the number of meibomian glands (Norn 1987); changes in meibomian gland morphology (Bron et al 1991) acinar atrophy, gland dropout and meibomian gland hyposecretion. (Arita et al 2008, Nien et al 2011); and displacement in Marx's line (Yamaguchi et al 2006). In addition, the lids may become less taut, which interferes with normal blinking (Blodi 1980). Furthermore, a lower amount of meibomian lipid on the lid margin was shown in females aged 20 to 29 compared with males, while the difference became indistinguishable in both genders over the age of 50 (Chew et al 1993).

Changes in the quality, quantity and appearance of meibomian gland secretion may also reflect changes to sex hormone activity in the gland. This condition might disrupt the function and stability of tears (Foulks & Bron 2003), leading to an increase in tear evaporation (Bron et al 2004, Foulks & Bron 2003).

A higher prevalence of meibomian gland disease was reported for men in a controlled age and gender cross-sectional study of 3280 Malay males (Siak et al 2012). This finding supports the influence of gender on meibomian gland dysfunction although there were no significant differences in meibomian gland assessment scores between genders shown in another study (Viso et al 2012).

Several clinical signs of dry eye were demonstrated to be different between genders. Lower tear break-up time (Cho & Yap 1993), lower tear volume measured with phenol red thread (Sakamoto et al 1993), higher tear evaporative rate (Guillon & Maïssa 2010), higher tear osmolarity (Farris et al 1986) and lower amount of secreted meibomian lipid (Chew et al 1993) were demonstrated in females. Such discrepancies may contribute to the higher prevalence of dry eye in females.

1.2.3 **Effects of Changes in Sex Hormone Levels in Females**

Increase in ocular symptoms; reduction in tear production and stability, reduction in ocular surface sensitivity and increase in ocular surface inflammation and ocular

surface dryness have been reported during the (luteal) oestrogen peak (Kiely et al 1983, Millodot & Lamont 1974, Riss et al 1982, Versura et al 2007) and during the final trimester of pregnancy (Doria et al 2006, Wong. J et al 2004). The receptor-hormone combination process is affected by the level of sex hormone binding globulin (SHBG)(Thijssen 1988). The level of SHBG increases in parallel to oestrogen and inversely to the level of progesterone which may be observed during the luteal phase of menstruation, pregnancy and hormone supplementation such as the use of oral hormone replacement therapy (Stomati et al 1996) and oral contraceptives. Reduced SHBG levels is understood to antagonise the effect of oestrogen (Jayaraman & Pike 2009, Kuba et al 2006). In addition the increase in oestrogen affects the SHBG level which reduces the amount of free testosterone and hence affects the androgen activation (Stomati et al 1996). SHBG binds to free testosterone and oestrogen and reduces their ability to interact with their receptors (Bachmann et al 2002, Thijssen 1988). Reduced androgen-receptor activation may decrease functioning of lacrimal and meibomian glands and adversely impact tear supply and stability. Consequently, increased symptoms of eye soreness, scratchiness, dryness, grittiness and burning were reported by 80% of pregnant women(Wong. J et al 2004).

The combination oestrogen-progesterone therapy was reported to improve symptoms and signs of dry eye (Affinito et al 2003, Altintaş et al 2004, Coksuer et al 2011, Guaschino et al 2003, Jung et al 2010, Kuscu et al 2003, Uncu et al 2006). This improvement is may be due to the ability of progesterone to prevent the impact of oestrogen alone in worsening the dry eye condition (Schaumberg et al 2001).

Oral contraceptives which mainly contain oestrogen may also increase the level of SHBG but reduce the level of free testosterone (Bancroft et al 1991) which may affect tear function and lead to contact lens intolerance (Brennan & Efron 1989, Chen et al 2013). However, two investigators did not find any effect on symptoms of ocular discomfort, tear film structure, non-invasive tear thinning time, evaporation rate, osmolarity, tear turnover rate, tear volume or tear protein levels in association with serum hormone changes induced by oral contraceptive use during normal cyclic variations (Feldman et al 1978, Tomlinson et al 2001). The investigators argued that their negative results could derive from variation in the types of contraceptive and duration of use.

Despite antagonising androgen-receptor activation, SHBG is expected to improve lacrimal gland function. Apart from the classical pathway (direct combination of androgens and their receptors), it is suggested that androgens may act by binding SHBG, which then binds its receptor (R SHBG). This in turn may activate cyclic adenosine monophosphateprotein (cAMP) kinase A and regulate protein transcription (Michels & Hoppe 2008) in the lacrimal gland, and improve its secretion.

Premature ovarian failure (POF) is defined as cessation of normal ovarian function in women younger than 40 (Smith et al 2004) who experience the same symptoms of oestrogen deficiency as postmenopausal women, including hot flushes and night sweats (Anasti et al 1998). Unsurprisingly, dry eye symptoms and worsening ocular surface integrity were also demonstrated in these patients (Smith et al 2004). Androgen deficiency may be responsible for the ocular surface disease or possibly due to a common genetic disorder in which there is dysfunction of a shared structural protein or other factor is required to maintain both developing ovarian follicles and a healthy ocular surface (Smith et al 2004). In contrast, polycystic ovary syndrome which is characterised by excessive levels of androgens, is also associated with ocular dryness (Bonini et al 2007).

Several sources of evidence indicate that changes in sex hormone levels could lead to dry eye during menopause. Ninety percent of those with Sjögren's syndrome, an autoimmune disease associated with lacrimal gland inflammation, meibomian gland dysfunction and severe dry eye, are women aged 40-50 (Porola et al 2007). These individuals had reduced levels of androgen, its metabolites and precursors (Sullivan et al 2003) and oestrogen (Forsblad-d'Elia et al 2009). A lower oestrogen and testosterone serum level was associated with poorer Ocular Surface Disease Index (OSDI) scores and tear function in postmenopausal women with dry eye (Gagliano et al 2014, Scuderi et al 2012). Furthermore, a large epidemiological study has revealed that exogenous oestrogen alone and a longer duration of hormone use were associated with a higher risk of dry eye in postmenopausal women (Schaumberg et al 2001). There is no clear consensus on whether oestrogen or androgen deficiency causes dry eye.

Complete androgen insensitivity syndrome (CAIS) is a condition in which the internal

female reproductive organs are missing due to an alteration in the androgen receptor gene, where the body responds to oestrogen but is insensitive to androgen (Brinkmann 2001, Warne 1997). The absence of androgen activity due to dysfunctional receptors may lead to dry eye in CAIS patients (Cermak et al 2003, Sullivan et al 2002b). Significant worsening of dry eye symptoms and signs in premature ovarian failure patients, has also been ascribed to androgen deficiency (Smith et al 2004). Furthermore, anti-androgen medication prescribed for prostatic indications is associated with meibomian gland malfunction, tear film instability and tear function irregularities although the scores of the symptoms remain unchanged (Krenzer et al 2000).

Androgen is likely to play an important role in normal homeostasis of the ocular surface while the majority of studies examining oestrogen have found no such association. The antagonistic effects between these sex hormones are important in maintaining the ocular surface homeostasis and protection. As changes to physiological levels of androgen, oestrogen and progesterone may affect ocular surface sensitivity, symptoms and signs, hormone replacement therapy (HRT) has been investigated as a potential remedy for dry eye (Adatia et al 2004, Affinito et al 2003, Akramian et al 1998, Altintaş et al 2004, Coksuer et al 2011, Guaschino et al 2003, Jung et al 2010, Sator et al 1998, Taner et al 2004) which is discussed further below.

1.3 Hormone Replacement Therapy and Dry Eye

Hormone replacement therapy (HRT) is a treatment comprising one or more female hormones, commonly oestrogen alone or combined with progesterone (Nelson et al 2002), for the relief of menopausal symptoms. The effects of HRT on dry eye signs and symptoms have been investigated in postmenopausal women and are displayed in Table 1.2. Relief from ocular discomfort and improvement in tear function have been reported in clinical studies where either oestrogen alone or the combination of oestrogen and progesterone or Tibolone (a synthetic oral HRT which has the effects of androgenic, oestrogenic and progesterone activity), was applied (Affinito et al 2003, Coksuer et al 2011, Guaschino et al 2003, Jung et al 2010, Sator et al 1998, Taner et al 2004). In contrast, HRT was one of the factors significantly associated with dry eye symptoms with the odd ratio of 1.69 (95% CI, 1.5-1.9) for oestrogen alone and 1.29

(95% CI, 1.1-1.5) for oestrogen plus progesterone/progestin in a large scale epidemiological study of 25,665 postmenopausal women (Women's Health Study)(Schaumberg et al 2001). This is consistent with the Blue Mountains Eye Study which suggested that HRT was among the factors significantly associated with self-reported dry eye symptoms with the odds ratio of 1.6 (95% CI, 1.0 - 2.5) in the elderly Australian population (Chia et al 2003). However, HRT was not shown to be among the medications related to dry eye in the Beaver Dam Eye Study subjects (Moss et al 2000).

In the Women's Health Study, the risk of dry eye symptoms increased with the duration of HRT use (Schaumberg et al 2001). Increased symptoms and reduced tear production were also reported in small clinical studies in which a combination of oestradiol and progesterone was used (Erdem et al 2007, Uncu et al 2006). Nevertheless, several other small clinical studies were unable to demonstrate changes in symptoms or tear function with HRT application (Kuscu et al 2003, Piwkumsribonruang et al 2010, Taner et al 2004).

1.3.1 **Factors Contributing to the Effectiveness of Therapy**

A good study design is required to obtain valid clinical trial results. The most valid clinical trials are prospective, randomised, controlled and double-masked (Asbell et al 2011). Randomised controlled trials assign participants to comparison groups to prevent selection bias. Non-randomised controlled trials are more prone to bias because the decision on the best treatment can be related to its prognosis and responsiveness, which may result in an inaccurate interpretation of the effect of an intervention (Kunz et al 2007).

Placebos allow discrimination of patient outcomes due to the test treatment from outcomes caused by other factors such as the natural progression of the disease (Jensen & Karoly 1991, Turner et al 1994). Double-masked procedures further minimise the risk of preference, so that both the subject and the examiner are unaware of the medication versus placebo. The effects that are absent in the placebo group can then be ascribed to the therapy. Unwanted placebo effects such as patients' expectations; a learning process associated with a patient's previous effective treatment; memory distortion; and the desire for symptom change may jeopardise the

results of non-placebo controlled studies (Jensen & Karoly 1991, Turner et al 1994).

The treatment duration must be sufficiently long to obtain the desired outcome (Asbell et al 2011). Longer duration of HRT was reported to contribute to the higher prevalence of dry eye symptoms (Schaumberg et al 2001). This means that an improvement in dry eye within a short duration may not necessarily justify the effectiveness of the therapy. It is therefore recommended for the therapy to have prolonged separate sessions after each observed improvement to adequately address any safety issues that the treatment regimen might create in a real-world setting (Asbell et al 2011).

The generally accepted minimum value of the power of the study is 80% with a sufficient sample size to appropriately address the efficacy of an intervention (Cohen 1988). A larger sample size increases the odds of real treatment differences being distinguished from chance variation (Machin et al 2011).

The inclusion of dry eye diagnosed subjects in clinical trials is recommended to determine the success of the intervention ,by comparing the validated objective and/or subjective clinical signs and symptoms relevant to ocular surface health before and after treatment (Asbell et al 2011). In addition, omitting washout or allowing concurrent medication may hamper detection of the effectiveness of the intervention (Ray 2003).

The hormone delivery route may affect the effectiveness of a therapy which can be applied orally or by transdermal application on the skin (as patches or gel) or in the eyes (as drops). Oral versus non-oral administration of HRT may induce different responses (Sitruk-Ware 2007). The advantages of non-oral administration include the avoidance of gastrointestinal and liver metabolism (Ligniers et al 1986). Increased liver production of oral SHBG occurs with exogenous oestrogen (Stomati et al 1996), which will bind to free testosterone, for instance, and reduce its rate of bioavailability. Transdermal and topical application allow the plasma level of the hormones to accurately reflect the actual dose delivered (Sitruk-Ware 2007). The non-oral route hence allows the hormone to enter the blood circulation directly and increase the chance of hormonal activity at the respective hormone receptors. HRT has been reported to improve dry eye symptoms and tear function through the application of topical oestrogen alone or the transdermal and/or oral combination of oestrogen and progesterone (Table 1.2) (Affinito et al 2003, Altintaş et al 2004, Coksuer et al 2011,

Guaschino et al 2003, Jung et al 2010, Sator et al 1998, Taner et al 2004), although two investigations found otherwise (Erdem et al 2007, Uncu et al 2006). A few studies showed no changes in either symptoms or signs (Piwkumsribonruang 2010, Taner2004, Kuscu 2003)**.**

Years since menopause affected the results of a study which compared between hormone therapy receivers and non-receivers where dry eye and non-dry eye subjects were included in both categories (Erdem et al 2007). The investigator reported a significant difference in menopause duration between dry eye and non-dry eye subjects in each category and proposed that a longer duration since menopause might increase the symptom.

It is important to establish circulating sex hormone levels prior to and after an intervention in order to confirm the alteration in hormone levels. The established hormone levels will ensure the absorption of the hormone treatment in the circulation.

Table 1.2 Studies on Dry Eye with Hormone Replacement Therapy in Postmenopausal Women

Investigator/ Study Design	Subjects	Type of Treatment	Route of Delivery	Duration of treatment	Significant Dry Eye Related Changes
Vavilis1997	1) 7 2) 4	E2 E2 +Pro	Transdermal Transdermal+ oral	4 months	↑cytological maturation changes in conjunctival epithelium in both groups
Sator 1998 (RCT)	1) 42 2) 42 controls	E2 placebo	Topical	4 months	↓symptoms ↑Schirmer in E2 receivers
Akramian1998 (RCT)	1) 11symptomatic 2) 11symptomatic (45-65years in both groups)	E2 placebo	Topical	one week	↓symptoms ↑ Schirmer& TBUT in E2 receivers
Marcozzi 2002	1) 8 2) 10	E2 E2 +Pro	Oral Transdermal	6 months	Lacrimal fluid peroxidise maybe regulated by E2
Affinito 2003 (RCT)	1) 25 2) 25 controls	E2 +Pro No treatment	Transdermal+ oral	3 months	↓symptoms ↑Schirmer
Guaschino 2003	1) 40 2) 40 controls	E2 +Pro No treatment	Oral	1 year	↑Schirmer
Kuscu2003	1) 10	E2 +Pro	Oral	6 months	No changes in symptoms, Schirmer, TBUT, corneal staining tests but ↓ MG inflammation
Altintas2004	1) 15 2) 24 age matched non-postmenopausal women controls	E2 +Pro No treatment	Oral	2 months	↑ Schirmer& TBUT
Taner 2004	1) 29 2) 25 3) 16 controls	Tibolone E2 +Pro No treatment	Oral Oral	6 months	↑ Schirmer&TBUT only in group 1 No changes in other groups
Uncu 2006	1) 19 2) 6 3) 5	E2+Pro Tibolone E2	Oral Oral Transdermal	12 months	↓ Schirmer in all subjects especially in group 3.

Investigator/ Study Design	Subjects	Type of Treatment	Route of Delivery	Duration of treatment	Significant Dry Eye Related Changes
Erdem2007	1) 20 DE & 20 NDE 2) 40 controls	E2 +Pro No treatment	Oral	3 months	↑number of DE patients
Piwkumsribonruang 2010 (RCT)	1) 21 DE 2) 21 DE controls	E2+Pro placebo	Transdermal+ oral	3 months	No significant changes in symptoms, Schirmer and TBUT
Jung 2010	1) 36	E2 +Pro	Oral	3 months	↑ Schirmer& TBUT, ↓in staining score & symptoms
Coksuer 2011	1) 34	E2 +Pro	Oral	6 months	↓OSDI ↑ Schirmer& TBUT
Scuderi 2012 (RCT Crossover)	1) 66 DE	Phytoestrogen /placebo	Oral	30 days	↓OSDI ↑ Schirmer& TBUT, ↓tear osmolarity

N.B

E2: Oestradiol
DE: Dry Eye Subjects
MG: meibomian gland
Pro: Progesterone
NDE: Non Dry Eye
OSDI: Ocular Surface Disease Index
RCT: Randomised control trial
TBUT: Tear break-up time

A limited number of studies listed in Table 1.2 conform to the prescription of a valid clinical trial. The non-conformity of the other studies might have led to discrepancies among their results. For instance, there were only five randomised controlled studies; four of which were placebo-controlled double-masked. In most studies reporting dry eye improvement, a combination of oestrogen and progesterone was prescribed rather than oestrogen alone.

Study duration may however influence the result of a well-designed study. A randomised, double-masked, placebo-controlled, crossover study with a 30-day washout period between study arms was conducted on 66 postmenopausal women with dry eye (Scuderi et al 2012). Symptoms and signs of dry eye improved in all subjects treated with phytoestrogens which are non-steroidal, diphenolic plant substances that have the capacity to bind to oestrogen receptors (Kuiper et al 1997). Phytoestrogens may enhance the androgenic effect with the elevation in testosterone level (Gunnarsson et al 2009) which allows the improvement in signs of dry eye as observed in this study. It was noted that the dry eye improvement was transient, with worsening of symptoms and signs observed during the washout period. The 30-day trial period may have been too short for the determination of longer-term adverse effects. A shorter treatment duration of one week (Akramian et al 1998) has also yielded an improvement in dry eye which may be in agreement with Scuderi's finding. However, a reduction in tear volume with oestrogen-alone therapy after twelve months, instead of six, (Uncu et al 2006) and worsening dry eye after a longer exposure to oestrogen therapy (Schaumberg et al 2001) may have determined the longer term adverse effects.

Tear volume and tear break-up time improved in dry eye subjects after six months of therapy with Tibolone (a synthetic oral HRT which has the effects of androgenic, oestrogenic and progesterone activity) in a study of 29 subjects (Taner et al 2004) compared to a significant reduction in tear volume in six Tibolone receivers after twelve months of therapy (Uncu et al 2006). Both study duration and sample size may have confounded the respective results.

With regards to delivery route, non-oral administration of HRT is preferable to oral since the hormones enter the blood circulation directly and increase the chance of hormonal activity at the respective hormone receptors (Sitruk-Ware 2007). This preference is supported by the improvement in symptoms and signs in oestrogen alone therapy via the topical route (eye drops) (Akramian et al 1998, Sator et al 1998). However, there are several studies demonstrating improvements in dry eye even with the oral intervention (Altintaş et al 2004, Coksuer et al 2011, Guaschino et al 2003, Jung et al 2010). Interestingly, these studies used a combination of oestrogen and progesterone as opposed to oestrogen alone. Progesterone may have mitigated the adverse effects of oestrogen alone (Jayaraman & Pike 2009, Schaumberg et al 2001).

Only a single study in Table 1.2 (Erdem et al 2007), compared the levels of circulating oestradiol before and after treatment, which helped to confirm the absorption of the treatment used.

HRT may have the potential to alleviate dry eye. However, validity of any intervention study depends on study design, including sample size, delivery route, duration since menopause and the establishment of hormone levels. It is highly recommended that a randomised controlled study with a proper study design be carried out to allow a better understanding of the potential relationship between sex hormones and dry eye symptoms and signs.

1.4 Measurement of Sex Hormone Levels, Ocular Symptoms, Ocular Surface Sensitivity and Clinical Signs

1.4.1 Measurement of Sex Hormone Levels

It is speculated that testosterone, oestrogen and progesterone reach all tissues in the body via blood circulation, but only exert an effect through their cognate receptors (Gupta et al 2005). Ninety seven to ninety nine percent of testosterone is bound to SHBG, leaving 1–3% readily available for physiological needs (Gauthaman & Ganesan 2008). Hence it is more appropriate to measure free testosterone level when identifying

any associations with dry eye indicators. In females, free testosterone concentration measured by equilibrium dialysis correlates well with calculated free testosterone (Bachmann et al 2002, Miller et al 2004) based on equations derived from the laws of mass action (Vermeulen & Giagulli 1991). When free testosterone is calculated using an online calculator (Vermeulen et al 1999), the concentration of circulating SHBG and total testosterone should be taken into account (Bachmann et al 2002).

The main source of androgen and oestrogen in postmenopausal women is through intracrinology (Labrie et al 2003) as described in section 1.1. A feasible method of measuring androgen levels is to identify the levels of its transformed metabolites and glucuronides such as 3α-diol G and androgen conjugated metabolites (ADTG) which diffuse into the general circulation and are the only route of elimination for androgens (Labrie et al 2006). 3α-diol G and ADTG in the circulation represent the level of testosterone at the peripheral site (Labrie et al 2006, Labrie et al 2003). DHEA-S is of key importance in the intracrinology process as it is the only source of sex steroid after menopause (Labrie 2010). In contrast, the ovary is proposed to be a site of an on-going testosterone production after menopause (Davison et al 2005). Two investigators have shown that testosterone levels were unaffected by age and were 40-50% lower in oophorectomised women than those in intact women throughout the 50-89 year age range (Fogle et al 2007, Laughlin et al 2000). However, the contribution of the ovary to the circulating pool of androgen after menopause is controversial since the enzymes for androgen biosynthesis were either absent or present in very low amounts in postmenopausal ovary (Couzinet et al 1989).

Oestradiol is the most potent natural oestrogen with a crucial role in the proliferation of normal breast and uterine cells (Luu-The & Labrie 2010). Oestradiol is a key regulator of growth, differentiation, and function in a wide array of target tissues, including the male and female reproductive tracts, mammary gland, and skeletal and cardiovascular systems (Hall et al 2001).Therefore, it is useful to confirm baseline oestradiol levels in premenopausal women as well as changes in hormone level with supplementation such as during hormone therapy in postmenopausal women.

The potential association between progesterone and symptoms and signs of dry eye has not been investigated although progesterone receptors are located on the lacrimal functional unit (Wickham et al 2000). In addition, progesterone is believed to be

effective in reducing the effect of oestrogen in combination therapy of oestrogen and progesterone since the use of oestrogen alone was associated with a higher risk than the combination of oestrogen and progesterone/progestin (Schaumberg et al 2001) (Table 1.2). The ratio of oestradiol to total testosterone concentration and the ratio of oestradiol to 3α-diol G concentration should also be considered since many of the unwanted effects of testosterone are actually caused by alterations in relative levels of these hormones (Murphy et al 2000, Rohr 2002).

Sex hormone levels can be measured in tears, saliva, urine and serum or plasma. Several methods have been used to measure androgen, its precursors and metabolites in blood; gas chromatography-mass spectrometry (GC-MS)(Labrie et al 2006, Stanczyk et al 2007); liquid chromatography-mass spectrometry (LC-MS)(Labrie et al 2006); equilibrium dialysis (Miller et al 2004) and immunoassays, including electrochemiluminescence immunoassay (ECLIA)(De Boever et al 1986), radioimmunoassay (RIA)(Miller et al 2004) and enzyme-linked immunosorbent assay (ELISA)(Moghassemi et al 2011).

The most widely used methods for measuring oestrogen in postmenopausal women are RIA and ECLIA (Blair 2010) although hormone levels in postmenopausal women are close to the limit of detection for these assays (Cauley et al 1991, McShane et al 1996). More sensitive RIA coupled with liquid chromatography currently provides the most sensitive and best validated immunoassay method for oestrone and oestradiol in serum in postmenopausal women (Blair 2010). However, this technique is costly and time consuming for the extraction and purification processes.

For measuring androgen and oestrogen levels, RIA, ELISA or ECLIA have the advantage of being technically simple, rapid, relatively inexpensive and allowing high throughput. However, the hormone concentration is often overestimated, results and reference intervals are not standardised or not well documented in different populations, and RIA generates radioactive waste (Rosner et al 2007).

Mass spectrometry in which multiple steroids can be measured in the same sample aliquot, offers a highly accurate hormone concentration reading if properly validated, and the technique is generally comparable with RIA after extraction and chromatography. However, mass spectrometry is relatively expensive, time consuming,

has a limited throughput, and the organic solvents used in the process require special facilities and waste disposal (Rosner et al 2007).

1.4.2 **Measurements of Ocular Symptoms in Dry Eye**

Assessment of symptoms and signs in dry eye has been carried out using questionnaires and objective tests, respectively. Dry eye is characterised by symptoms of discomfort and poorer outcomes measures in objective tests (Afonso et al 1999, Cennamo et al 2007, Johnson 2009, Macri & Pflugfelder 2000, Macri et al 2000, Sade de Paiva et al 2003). However, it is well accepted that there are often no associations between symptoms and signs of dry eye (Nichols et al 2004, Sullivan et al 2014).

Ocular symptoms questionnaires are used to grade the severity of ocular discomfort (Begley et al 2002c, Johnson & Murphy 2007, Simpson et al 2008, Solomon et al 2008, Vitale et al 2004), to discriminate dry eye subjects from normal subjects (Johnson 2009), to measure the impact of dry eye on quality of life and vision function, and to assess the effects of intervention and understand the risks of treatment (Guillemin et al 2012). Dry eye symptoms range from mild, transient irritation to persistent dryness, burning, itchiness, redness, pain, ocular fatigue and visual disturbance (Lee et al 2002). Among the questionnaires utilised are Subjective Evaluation of Symptom of Dryness (SESoD) (Simmons et al 2003), Women's Health Study (Schaumberg et al 2001), Dry Eye Questionnaire (DEQ)(Begley et al 2002c), Ocular Surface Disease Index (OSDI) (Miller et al 2010) and Ocular Comfort Index (OCI) (Johnson & Murphy 2007).

The Women's Health Study questionnaire has been used to assess the prevalence of dry eye among women, based on the current status of dryness and irritation symptoms and the previous history of clinically diagnosed dry eye, and was used in key epidemiological and clinical studies of dry eye (Schaumberg et al 2001, Schaumberg et al 2009, Schaumberg et al 2003, Uchino et al 2008).

The OSDI is one of the most widely used ocular symptom questionnaires in dry eye studies, including some involving dry eye and HRT as discussed in Table 1.2 (Coksuer et al 2011, Scuderi et al 2012). The OSDI was designed to assess symptoms of ocular irritation and their impact on vision-related function and to grade the severity of dry eye

(Guillemin et al 2012, Ozcura et al 2007). It was also able to show treatment benefits in dry eye (Chang et al 2009, Russo et al 2007, Stevenson et al 2000,Yüksel et al 2010). The OSDI also has good reliability, validity and has proven to be a good ocular comfort indicator in postmenopausal women with dry eye (Srinivasan et al 2008). However, it is not well targeted to patients with dry eye disease who were diagnosed using the Women's Health Study questionnaire (Dougherty et al 2011).

The Ocular Comfort Index was developed to measure the severity of discomfort caused by ocular surface disease. The OCI has good repeatability, reliability and validity; it was designed and validated using Rasch analysis and construct validity has been demonstrated (Johnson & Murphy 2007). However, it has not yet been used in a postmenopausal women cohort.

The Dry Eye Questionnaire (DEQ) was developed to quantify and characterise the frequency of ocular symptoms and their diurnal intensity in dry eye patients (Begley et al 2002b, Chalmers et al 2010,Simpson et al 2008). The DEQ has been shown as an effective tool for categorising patients based on symptom severity and may be useful in treatment trials in elderly populations (Kim et al 2011). The DEQ 5 is a screening test that generates a score using five questions from DEQ covering frequency of watery eyes, frequency, and late day intensity of discomfort and dryness (Chalmers et al 2010). However there are no published reports of its use in hormone related studies.

In the Subjective Evaluation Symptom of Dryness (SESoD), a single question on the severity of dryness is shown to be repeatable and effectively used to segregate symptomatic and asymptomatic groups. However, there are no published reports of its use in hormone related studies.

Numerical Rating Scales have not been previously used in studies of dry eye but they are a reasonable alternative to the visual analogue scale as a method of assessing subjective visual quality (Papas & Schultz 1997). Since the rating scale is interpreted in ascending or descending order, for instance from least to most comfort, the scale is applicable for non-visual symptom assessment.

In Table 1.2, apart from the OSDI, other ocular comfort questionnaires included are the Visual Analogue Scale (VAS) (Affinito et al 2003, Piwkumsribonruang et al 2010, Sator et al 1998); severity scale of a group of symptoms consisting of foreign body and

burning sensation, tearing, presence of mucoid secretion and redness (Kuscu et al 2003); severity scale of irritation and pain sensation (Akramian et al 1998) and severity scale of an awareness of dryness, tearing, injection, stinging, blurring, straining, foreign body sensation, photophobia, itching and headache (Jung et al 2010). Erdem et al (2007) used symptoms of dryness, itching, foreign body sensation, tearing and photophobia in measuring dry eye before and after treatment. Ocular symptoms are the major concern for postmenopausal women with dry eye. Therefore, it is important for investigators to utilise appropriate validated questionnaires in defining the issue.

1.4.3 **Measurements of Ocular Surface Sensitivity in Dry Eye**

Aesthesiometry is an important ocular surface health indicator since it has the ability to detect disruption in sensory function as occurs in dry eye (Barboza et al 2008, Benitez-del-Castillo et al 2001, Bourcier et al 2005, De Paiva & Pflugfelder 2004, Situ et al 2008b, Toker & Asfuroglu 2010, Tuisku et al 2008, Versura et al 2006, Xu et al 1996). The Cochet-Bonnet is an example of a widely used contact aesthesiometer that stimulates mechanosensory receptors. Based on Von Frey's concept (1894), this instrument consists of a fine nylon filament of either 0.08 mm or 0.12 mm in diameter that is of adjustable length so that different intensities of stimulus can be applied. This instrument applies force to the ocular surface that is inversely proportional to the nylon filament length.

Although Cochet-Bonnet is considered the gold standard aesthesiometer, several limitations exist, such as a truncated stimulus range, imprecise and poor repeatability of location of the stimulus on the ocular surface, patient awareness and disruption of the epithelial surface(Golebiowski et al 2005, Golebiowski et al 2011, Millodot 1967, Murphy et al 1996). In order to overcome these limitations, non-contact aesthesiometers such as the non-contact corneal aesthesiometer (NCCA)(Murphy et al 1996), Belmonte aesthesiometer (Belmonte et al 1999) and the CRCERT- Belmonte aesthesiometer (CBA)(Golebiowski et al 2013) were introduced. Non-contact or pneumatic gas aesthesiometers are able to stimulate mechanical, chemical or thermal receptors in the ocular surface by changing the intensity, type and duration of gas and temperature delivered. The Belmonte Ocular Pain Meter (BOPM) by Deriva Global (Valencia, Spain) is the latest commercially available non-contact aesthesiometer

which aims to stimulate neuro receptors of the ocular surface. As with the other non-contact aesthesiometers, stimuli comprise pulses of medical quality air to the ocular surface.

In dry eye, both hypersensitivity and hyposensitivity of the ocular surface have been reported (Adatia et al 2004, Barboza et al 2008, Belmonte et al 1999, Benítez-del-Castillo et al 2007, Bourcier et al 2005, De Paiva & Pflugfelder 2004, Han et al 2010, Situ et al 2008b, Toker & Asfuroglu 2010, Tuisku et al 2008, Versura et al 2006, Xu et al 1996). This apparent conflict is likely to be due to differences in instrumentation, specifically stimulus characteristics, with hypersensitivity reported with the modified Belmonte aesthesiometer (Sade de Paiva et al 2003, Situ et al 2008b, Tuisku et al 2008) and hyposensitivity with the Cochet-Bonnet (Adatia et al 2004, Han et al 2010, Toker & Asfuroglu 2010, Versura et al 2006, Xu et al 1996) instrument.

Changes at the sensory nerve ending may lead to either increase or reduction in ocular surface sensitivity as described below. Disruption to the corneal epithelial barrier function (Sade de Paiva et al 2003) and damaged sensory nerve endings (Benítez-del-Castillo et al 2007, Bourcier et al 2005, Sade de Paiva et al 2003, Toker & Asfuroglu 2010, Xu et al 1996) may modulate ocular surface sensitivity in dry eye. Greater access of environmental stimuli to the end of the sensory nerve is speculated to result in ocular surface hypersensitivity (Sade De Paiva & Pflugfelder 2004). Ocular irritation in dry eye is accompanied by an unstable tear film which leads to drying of the surface. This condition produces a mechanical distortion of the epithelium (corneal epithelial barrier) and loss of membrane mucin coating and interconnecting tight junctions resulting in interrupted intercellular spaces where nerve endings are located (Belmonte et al 2004). Therefore, greater access of environmental stimuli to the corneal sensory receptors is feasible and hypersensitivity is reported by dry eye patients (Sade De Paiva & Pflugfelder 2004).

Conversely, hyposensitivity may occur due to an adaptation of corneal sensory nerves (Stapleton et al 2013) where the frequency and intensity of action potentials may decline during adaptation. Eventually the sensitivity for pain reduces, resulting in a stronger stimulation requirement to elicit corneal sensation in dry eye patients (Xu et al 1996).

Contact and non-contact aesthesiometers are different in stimulus composition, temperature and area of stimulation and these aesthesiometers measure different aspects of the neural response (Belmonte et al 1999, Golebiowski et al 2011). Nevertheless, an increase in symptoms is mostly reported in either hypersensitivity or hyposensitivity of the ocular surface in dry eye.

There are limited studies on conjunctival sensitivity. The threshold varies across the conjunctiva with a higher sensitivity at the superior compared to the inferior bulbar conjunctiva as measured with the 0.12 mm nylon thread of the Cochet-Bonnet (Norn 1973). Conjunctival threshold was also measured with the non-contact aesthesiometers (Golebiowski et al 2008, Situ et al 2008a, Stapleton et al 2004) where corneal sensitivity was higher than conjunctival and their measurements were associated with each other. Conjunctival sensitivity was positively associated with tear volume and tear break-up time, and negatively associated with symptoms as measured with COBO (Toker & Asfuroglu 2010). In contrast, conjunctival sensitivity was positively associated with symptoms with the non-contact aesthesiometer in a sample including both symptomatic and asymptomatic dry eye subjects (Situ et al 2008a). These findings confirmed the relevance of conjunctival sensitivity measurement in dry eye studies. Furthermore, conjunctival and corneal sensitivity to pneumatic cool stimulation increased in subjects with symptoms of ocular dryness, and this hyperesthesia seems to be more significant in the conjunctiva (Situ et al 2008b).

Table 1.3 lists investigations of the ocular symptoms and surface sensitivity in dry eye. Hyposensitivity was reported in all COBO and original Belmonte aesthesiometer-based studies, as opposed to hypersensitivity in all studies using CRCERT-Belmonte aesthesiometer (CBA) which may be due to the differences in instrument design. The CBA is able to stimulate the precise mechanical receptors in the cornea and conjunctiva which allows the sensitivity of the cornea and conjunctiva to mechanical stimuli to be determined (Golebiowski et al 2005). In the original Belmonte, it is likely that some temperature sensitive “cold” nociceptors on the cornea and thermosensitive neurons in the conjunctiva are likely to be recruited inadvertently which resulted in a higher sensitivity measurement (Golebiowski et al 2011).

Based on the current observation, the type of aesthesiometer influences the sensitivity measurement. Therefore, the actual ocular surface sensitivity measurement may not

only depend on the level of oestrogen and androgen but also the type of aesthesiometer used.”

Table 1.3 Relationships between Ocular Surface Sensitivity and Symptoms in Dry Eye

Investigator	Subjects	Aesthesiometer used	Symptoms Questionnaire	Results
Xu et al(1996)	1. 15 SDE 2. 18NSDE 3. 26 Control	COBO	Symptoms questionnaire	CS↓ Symptoms ↑ in SDE and NSDE than control
Adatia et al (2004)	1. 18 SDE	COBO	OSDI	CS↓ Symptoms ↓ (insignificant)
Versura et al (2007)	1. 62 Primary SDE 2. 56NSDE 3. 59 non autoimmune disease DE	COBO	OSDI	CS↓ Symptoms ↑ in all groups
Barboza et al (2008)	1. 17 SDE 2. 25 Normal	COBO	OSDI	CS↓ Symptoms ↑ in SDE
Han et al (2009)	1. 20 SDE 2. 20 NSDE	COBO	OSDI	CS↓ Symptoms ↓ (insignificant)
Toker et al (2010)	1. 23 SDE, 2. 14 NSDE, 3. 35 Control	COBO	OSDI	CS↓ Symptoms ↑in DE
Bourcier et al (2005)	1. 14 SDE 2. 30 NSDE 3. 42 Control	Original Belmonte	3 Symptoms Questionnaire	CS & CJS ↓ Symptoms ↑ in DE
Benitez-del-Castillo et al (2007)	1. 10 SD 2. 11 NSDE 3. 20 Control	Original Belmonte	Symptoms Questionnaire	CS↓ Symptoms ↑in DE
Situ et al (2008)	1. 43 DE 2. 54 NDE	CBA	OSDI	CS & CJS ↑ Symptoms ↑ in DE
De Paiva&Pflugfelder (2004)	1. 20 DE 2. 20 NDE	CBA	11 Symptoms Questionnaire	CS↑ Symptoms ↑ in DE
Tuisku et al (2008)	1. 20 SDE 2. 10 NSDE	CBA	OSDI	CS↑ Symptoms ↑ in SDE

CS: Corneal Sensitivity
CJS: Conjunctival Sensitivity
DE: Dry Eye
NDE: Non Dry Eye
SDE: Sjögren Dry Eye
NSDE: Non Sjögren Dry Eye
OSDI: Ocular Surface Disease Index
COBO: Cochet-Bonnet
CBA: Modified CRCERT Belmonte Aesthesiometer

1.4.4 **Measurements of Clinical Signs of Dry Eye**

The importance of adequate sex hormone levels on the functions of lacrimal and meibomian glands were investigated based on the volume (Schirmer), osmolarity and stability of tears [tear break-up time (TBUT)] (Scuderi et al 2012, Gagliano et al 2014, Mathers et al 1998).

The Schirmer test is still commonly used to evaluate aqueous tear production ever since it was first described by Schirmer in 1903, despite its low reproducibility, sensitivity and specificity; and frequent discomfort as reported by patients (Cho & Yap 1993). This frequent discomfort is later reduced with the usage of a fine cotton thread, impregnated with phenol red dye, known as " phenol red thread " (PRT) (Hamano et al 1983). However, the advantages of PRT over the Schirmer test are still controversial (Yokoi et al 2000, Tomlinson et al 2001).

Tear osmolarity is a single test that is able to capture the balance of inputs and outputs from the tear film dynamics (Tomlinson et al 2006) and is regarded as the signature feature that characterizes the condition of ocular surface dryness (Lemp et al 2007). Tear osmolarity has commonly been measured by observing the changes in the freezing point of tear samples (Savini et al 2008). This technique has evolved into the temperature-corrected impedance measurement with the usage of the TearLab osmolarity, to provide an indirect assessment of osmolarity (range from 275–400 mOsms/L) (Versura et al 2009).

TBUT is defined as the interval following a blink to the first occurrence of dry spots on the cornea (Norn 1969), which signifies the tear film stability (Nichols et al 2003). This interval can be measured invasively after instilling fluorescein dye to detect the breaks in tears (TBUT) or noninvasively by observing the reflection of a grid pattern from the tear film surface (Johnson & Murphy 2005). An unstable tear film may indicate the presence of ocular irritation due to reduced aqueous tear production or an increase in tear evaporation, as in the case of Meibomian gland dysfunction (Savini et al 2008).

Fluorescein sodium has been used to detect corneal epithelial defects and which are usually seen in the lower third of the cornea and then may spread over the entire corneal surface (Savini et al 2008). In dry eyes, fluorescein staining may also be seen on the conjunctival surface and conjunctival damage precedes that of the cornea and is more severe (Yokoi and Kinoshita 1998). However, detecting fluorescein staining on the conjunctival epithelium can be more difficult because of the poor scleral contrast which may be overcome with Lissamine green (LG) where ocular surface staining intensity can be better appreciated with a yellow (blue-free) barrier filter (eg, Wratten 12 yellow) used in front of the ocular eyepieces (Koh et al 2003).

The Schirmer test result is strongly associated with TBUT and fluorescein staining in dry eye, suggesting that dessication of ocular surface occurs as a result of compromised tear volume (Nichols et al 2003, Pflugfelder et al 1998). These findings suggested that apart from tear function, ocular surface integrity should also be considered when investigating the effect of sex hormones on dry eye.

Changes in the quality and quantity of Meibomian gland secretion may also be indicators of disruption to the hormone receptors activity of the gland. These changes might disrupt the functions and stability of tears (Foulks & Bron 2003), leading to an increase in tear evaporative dry eye (Bron et al 2004, Foulks & Bron 2003). Several grading systems have been used to diagnose Meibomian gland dysfunction (Bron et al 1991, Mathers & Lane 1998, Mathers et al 1991, Pflugfelder et al 1998, Shimazaki et al 1998) based on the Meibomian gland dropout, altered secretion and changes in lid morphology (Tomlinson et al 2011).

It is important to conduct the relevant clinical tests to identify the effects of sex hormone therapy on dry eye since no study has actually probed into these direct associations, which may be present between circulating sex hormone levels and these clinical signs.

1.5 Gaps in the Literature

There are several gaps in the literature in the understanding of hormone levels and dry eye signs and symptoms and ocular sensitivity. In dry eye clinical signs, Schirmer and tear break-up time (TBUT) tests were observed in response to HRT (Table 1.2).

However, only one study reported the associations between these parameters and the levels of the respective hormone used, which were mainly oestrogen and the combined oestrogen and progesterone (Scuderi et al 2012). To date, there are only three reports of the associations between the circulating level of oestradiol (Mathers et al 1998) and testosterone with tear osmolarity in postmenopausal women (Scuderi et al 2012, Gagliano et al 2014). The studies in this thesis investigated the associations between the circulating levels of oestradiol; testosterone; progesterone; sex hormone binding globulin; and the androgen metabolites and tear function, ocular surface integrity; and Meibomian gland assessment in a normal population of either gender and not just the postmenopausal women.

Progesterone mRNA receptors are located on the ocular surface (Wickham et al 2000). Combined progesterone and oestrogen HRT therapy demonstrated an improvement in dry eye (Schaumberg et al 2001) that might be caused by progesterone mitigating against impact of oestrogen (Jayaraman & Pike 2009, Kuba et al 2006). Furthermore, progesterone is proposed to play a role in pain sensation modulation (Romano et al 1988). However, there is no published evidence of any association between progesterone level and dry eye and ocular surface sensitivity. Identifying such evidence may clarify the function of progesterone in relation to dry eye.

Only two retrospective studies showed positive effects in dry eye with the combined oestrogen and testosterone treatment (Scott et al 2005, Nanavaty et al 2013). However, this effect has not been broadly investigated in an interventional study.

HRT may have the potential to alleviate dry eye and this is supported by several related interventional studies as displayed in table 1.2. However, only a few applied the recommended study design and none investigated the effect of androgen treatment as a potential remedy for dry eye. Therefore, an appropriate interventional study of HRT is needed to determine the effects of androgen on symptoms and clinical indicators of dry eye.

1.6 Conclusion

The associations between both dry eye symptoms and ocular surface sensitivity with sex hormone (androgen, oestrogen and progesterone) levels, particularly in a normal

population of both genders, have not been investigated although such associations are important to explain the impact of age and gender on the relationship between dry eye and sex hormones. Furthermore, hormone levels are affected by these two factors.

With the contradictory expectations for the role of oestrogen in modulating somatic sensitivity, it would be relevant to identify the impact of changes in sex hormone levels on ocular sensitivity and symptom reporting.

Given the evidence for hormones modulating meibomian and lacrimal gland functions, an appropriate interventional study of HRT is required to elucidate the link between sex hormones and symptoms; and signs of dry eye. Such study may lead to the identification of the specific sex hormones or metabolites and precursors that play a role in the aetiology of dry eye. These data may eventually contribute to the development of an alternative treatment for dry eye.

1.7 Thesis Aims

1) To identify the relationships between levels of circulating sex hormones and ocular symptoms, ocular surface sensitivity and clinical indices of dry eye.

2) To investigate the effects of sex hormone treatment on ocular symptoms, ocular surface sensitivity and clinical indices of dry eye in postmenopausal women with dry eye.

1.8 Thesis Hypotheses

1) In a normal population dry eye symptoms are associated with a lower androgen level and a higher oestrogen level.

2) In a normal population of males; and menstruating and postmenopausal women, dry eye symptoms are associated with a lower androgen level and a higher oestrogen level.

3) In postmenopausal women with dry eye symptoms:

i. Serum levels of androgen are lower but serum levels of oestradiol are higher. Lower androgen and higher oestradiol are associated with an increase in symptoms, a decrease in ocular surface sensitivity and greater clinical signs of dry eye,

ii. Testosterone and the combination supplement of testosterone and oestradiol reduce the symptoms, increase the surface sensitivity, and reduce/improve the clinical signs of dry eye,

iii. Oestradiol supplementation increases the symptoms, decreases the surface sensitivity, and worsens the clinical signs of dry eye.

CHAPTER 2

Methods Development

Calibration and Repeatability of the Cochet-Bonnet Aesthesiometer and its Comparison to the Belmonte Ocular Pain Meter

2.1 Introduction

Reduced ocular surface sensitivity during the oestrogen peak (luteal phase of menstrual cycle) (Millodot & Lamont 1974, Riss et al 1982) and pregnancy (Millodot 1977b) suggests the influence of sex hormone levels on the parameter. Sensitivity can be measured with an aesthesiometer which delivers a variable stimulus to the ocular surface.

Ocular surface sensitivity measurement is dependent on the characteristics of the stimulus and the type of aesthesiometer selected. The Cochet-Bonnet aesthesiometer (COBO) is the most commonly used aesthesiometer and is considered the gold standard instrument in ocular surface sensitivity measurement. However, the COBO has several limitations and gas aesthesiometers were introduced to overcome these limitations, as described in section 1.4.3. Nevertheless, the stimuli delivered by the gas aesthesiometers as Non-Contact Corneal Aesthesiometer (NCCA) (Murphy et al 1996) and the CRCERT Belmonte aesthesiometer (CBA) (Golebiowski et al 2013) differ in that they are not purely mechanical as is the COBO stimulus.The Belmonte Ocular Pain Meter (BOPM) by Deriva Global (Valencia, Spain) is a commercially available non-contact aesthesiometer which aims to stimulate either the mechanical or chemical or thermal receptors of the ocular surface (Figure 2.1). As with the other non-contact aesthesiometers, pulses of medical quality air are delivered to the ocular surface and given that the BOPM is a new instrument, it is important for its repeatability to be established.

The COBO delivers only a mechanical stimulus, where the stimulus is exerted on a small fixed area by the tip of the nylon filament (Figure 2.2). The exerted pressure is measured in g/mm^2 and periodic calibration of the instrument is required as the filament ages, and humidity and degree of use influence the pressure (Millodot 1967, Murphy et al 1998). Although the COBO is most widely used, the instrument's repeatability has not been previously published.

This chapter examines the potential use of the BOPM to reliably measure ocular sensitivity. The sections following are describing its repeatability; comparing it with the COBO and discussing the calibration and repeatability of the COBO in subjects without ocular surface disease.

Figure 2.1 The Belmonte Ocular Pain Meter (BOPM manual, DerivaGloba,Valencia, Spain)

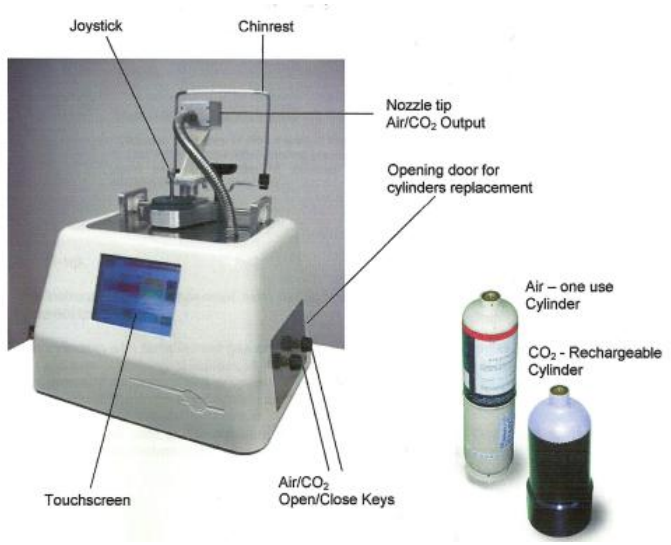


Figure 2.2 The Cochet-Bonnet aesthesiometer (COBO) by Luneau Ophthalmologie, Chartres, Paris, France

2.2 Aims

1. To determine the repeatability of the BOPM for corneal and conjunctival sensitivity measurement.
2. To compare the corneal and conjunctival sensitivity measurement between the BOPM and the COBO.
3. To determine the repeatability of the COBO for corneal and conjunctival sensitivity measurement.

2.3 Repeatability of the Belmonte Ocular Pain Meter (BOPM)

2.3.1 Aim

To determine the repeatability of the BOPM for ocular surface sensitivity measurement

2.3.2 **Method**

2.3.2.1 Study Design

Study subjects were measured twice, approximately 24 hours apart. Thresholds of the central cornea and the inferior conjunctiva of the right eye were measured at the same time of day between 11 A.M and 5 P.M to limit the impact of diurnal variability.

2.3.2.2 Subjects

The age range of the normal 23 (20F:3M) study subjects was 21 - 32 (mean 27 ± 7) years. A general ocular surface assessment was performed to exclude subjects with ocular surface diseases.

Inclusion criteria

At least 18 years old

Exclusion criteria

Subjects without ocular surface disease

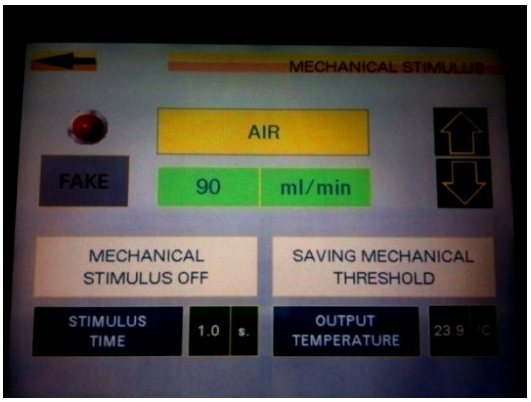
The sample size was calculated based on the standard deviation of corneal threshold from a previous study (Golebiowski et al 2005). This study used the CRCERT Belmonte aesthesiometer to determine the repeatability of an unequal staircase technique (Garcia-Perez Staircase) in measuring corneal mechanical threshold (Golebiowski et al 2005, Garcia-Perez 2001).This technique was also applied in the current study. Twenty three subjects without ocular surface disease were required to detect a difference of 21.5 ml/min between the two measurements of corneal threshold at a significant level of 95% and the power level set to be 0.80.

Subjects were recruited from the School of Optometry and Vision Science (SOVS) via email and from the University of New South Wales campus and general community via flyers. Ethics approval was obtained from the Human Research Ethics Advisory panel at the University of New South Wales, Sydney, Australia and followed the tenets of the Declaration of Helsinki (HREA 10025). Signed informed consent was obtained from each subject prior to enrolment in the study (Appendix 1).

2.3.2.3 Procedure

Figure 2.3 Operation of the BOPM

Figure 2.4 The BOPM touch screen



The subject was seated in front of the instrument with the head firmly pressed against the forehead and chin-rest (Figure 2.3). The subject's lateral canthus was aligned (vertically) with the exit gas nozzle. The nozzle was moved towards the centre of the cornea by controlling the joystick until it reached the working distance of 5mm as recommended in the instruction manual. This distance was determined from the video of a magnified ruler scale displayed on a computer monitor, which was captured by a video camera attached to the BOPM. This video also aided the alignment of the stimulus with the corneal apex (central cornea) and to the inferior conjunctiva, 2 mm vertically below a tangent to the inferior limbus.The BOPM stimulus type, duration and temperature are controlled by manipulating an electronic touch screen (Figure 2.4).The temperature of the air exiting the nozzle was set to 50°C to give an on eye temperature of 34ºC, equivalent to cornea's temperature (Efron et al 1989). The technique was demonstrated to the subject and sham stimuli were applied to check for false positive responses on the left eye prior to the experiment.

Subjects were asked to fixate on distance targets arranged in a grid pattern at 1 m from the contra lateral eye. They were asked to focus at the centre of the grid when measuring central corneal sensitivity, and elevate their eyes to focus at the spot target aligned and separated by 7 cm vertically above the centre of the grid when measuring the inferior conjunctival sensitivity. They were requested to blink once and were then immediately presented with a stimulus and requested to respond with "yes" or "no" to whether they had felt the stimulus. The stimulus duration was one second and there

was a 20-second break between stimuli. Subjects were asked to alternate their fixations between the two targets. The central cornea was chosen as an easy location for stimulus placement in this study and for comparison with previous work. The inferior conjunctiva was selected as being susceptible to the effects of tear film instability in dry eye disease and for comparison with previous studies using an air jet aesthesiometer (Stapleton et al 2004). No other study has measured either the upper, nasal or temporal conjunctiva with an air jet aesthesiometer on subjects that are not contracted with ocular surface disease.

A starting stimulus of 200 ml/min was delivered using the Garcia–Perez staircase technique (García-Pérez 2001). The instrument settings were at 5ml/min for each step. Then, unequal ascending (10 ml/min) and descending (5 ml/min) steps of stimulus magnitude were used to obtain a reversal in subjects' subjective responses. Threshold was calculated from the mean of the final six reversals.

2.3.3 **Statistical Analysis**

Data normality was assessed using the Shapiro-Wilk test ($p>0.05$). Differences between visits were evaluated using a paired t-test ($p<0.05$). Bland and Altman graphs were plotted from the means and difference between the means of the two sets of threshold measurements (Bland & Altman 1986). The coefficient of repeatability (CoR) was defined as 1.96 times the within-subjects standard deviation (1.96 SD) and limits of agreement (LoA) were defined as the bias ± (1.96 SD). Bias was calculated from the mean of the differences between both sets of measurements.

2.3.4 **Results**

The age range of the normal 23 (20F:3M) study subjects was 21 - 32 (mean 27 ± 7) years. A general ocular surface assessment was performed to exclude subjects with ocular surface diseases.

Measurements were taken in a room with an ambient humidity range of 38-46% and a temperature of 23 - 25ºC. Corneal and conjunctival thresholds were normally distributed. The paired t-test showed no significant differences between two visits at either location.

Table 2.1 The Means and Standard Deviations of Corneal and Inferior Conjunctival Thresholds by Visit (24 hours apart) and Location (n=23).

	Central Corneal Threshold	Inferior Conjunctival Threshold
	Mean ± SD (ml/min)	
Visit 1	77.2 ± 44.0	85.1 ± 45.1
Visit 2 (24 hours later)	72.0 ± 39.5	74.6 ± 44.8
p value	0.40	0.16

No significant difference of theresholds were observed between visits

Bland and Altman plots indicate the LoA and bias values for the central cornea and inferior conjunctiva thresholds, respectively (Figures 2.5 and 2.6).The bias for corneal thresholds was -5.3 ml/min. The CoR of ±57.3 ml/min indicates that 95% of the differences between the two visits could be expected to lie between +52.1 and -62.6 ml/min. The bias for the inferior conjunctival threshold was -10.5ml/min. The CoR of ±69.3 ml/min indicates that 95% of the differences between the two visits could be expected to lie between +58.8 and -79.7 ml/min.

Figure 2.5 Difference between thresholds of the first and second visits (24 hours apart) plotted against their means for the central corneal threshold (CCThd). The dotted line represents a bias of -5.3ml/min. The dashed lines represent the Limits of Agreement of +52.1 and -62.6 ml/min and Coefficient of Repeatability of 57.3 ml/min.

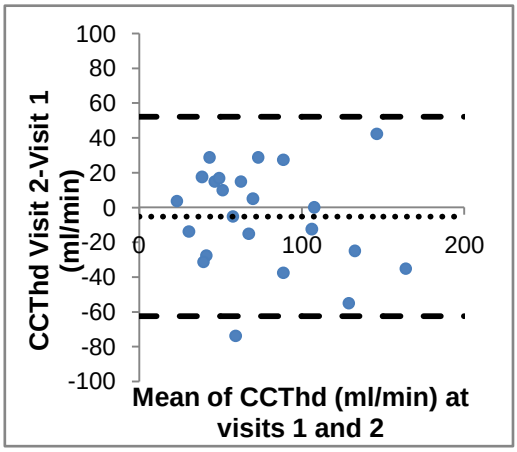
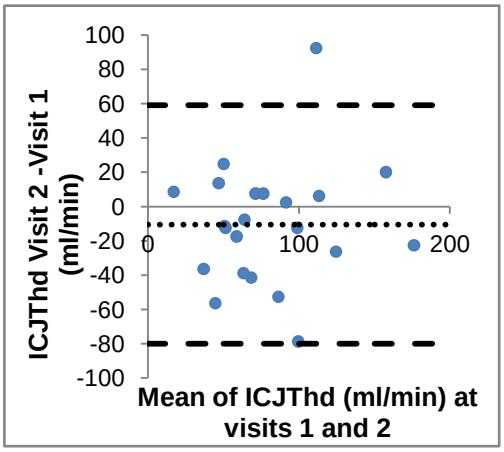


Figure 2.6 Difference between thresholds of the first and second visits (24 hours apart) plotted against their means for the inferior conjunctival threshold (ICJThd). The dotted line represents a bias of –10.5ml/min. The dashed lines represent the Limits of Agreement of +58.9 and –79.7 ml/min and Coefficient of Repeatability of 69.3 ml/min.



2.3.5 **Discussion**

Our results indicated that measurements of corneal and conjunctival sensitivities in subjects without ocular surface disease do not differ between 24 hour visits. Both of the coefficient of repeatability for central cornea (± 57.3 ml/min) and inferior conjunctiva (± 69.3 ml/min) thresholds were substantially higher than the published values which were ±18.3 ml/min and ± 29.4 ml/min respectively using a different non-contact aesthesiometer (Golebiowski et al 2005, Stapleton et al 2004).The previous studies were performed on subjects without history of ocular pathology or systemic disease.

A high coefficient of repeatability with the BOPM may be due to differences in instrument features. Poor reproducibility of the BOPM stimulus was subsequently demonstrated (Lum 2013). Specifically, the instrument was incorrectly calibrated for airflow rate, area of the stimuli and temperature. In addition, there was a large variation in the force exerted by the air jet stimulus. Furthermore, the footprint (cross sectional area) size of the BOPM stimulus was larger than either the CBA or NCCA footprint (Golebiowski et al 2005, Murphy et al 1996). In conclusion, due to the poor reproducibility of the BOPM stimulus, the instrument is not recommended for ocular surface threshold measurement.

2.4 Comparison of the Cochet-Bonnet (COBO) and the Belmonte Ocular Pain Meter (BOPM)

2.4.1 **Aim**

To compare the force exerted by the COBO and the BOPM.

2.4.2 **Method**

2.4.2.1 Study Design

Thresholds of the central cornea and the inferior conjunctiva of the right eye were measured once between 11 A.M and 5 P.M.

2.4.2.2 Subjects

A sample size of eighteen subjects was calculated based on the standard deviation of the force on the cornea from a previous study (Golebiowski 2005)to detect a difference of 0.2 mN between the BOPM and COBO at a significant level of 95% and the power level set to be 0.80. Since the current study and the BOPM repeatability study described in Section 2.3 were performed at the same time, the ethics approval from Human Research Ethics Advisory panel (HREA10025) and subject recruitment were as previously described in section 2.3.2.2. Signed informed consent was obtained from each subject prior to enrolment in the study (Appendix 1).

The age range of subjects (15F:3M) in the study was 21-32 (mean 25.0 ± 4.4) years. A general corneal assessment was performed to exclude subjects with ocular surface diseases.

Inclusion criteria
At least 18 years old
Exclusion criteria
Subjects without ocular surface disease

2.4.2.3 Procedure

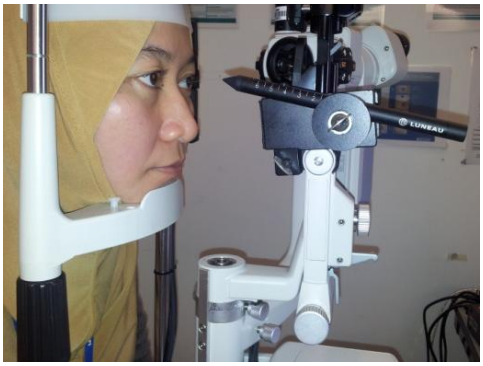
The BOPM measurement procedure is described in section 2.3.2.3 while the COBO measurement procedure is described below. The COBO and BOPM were used alternately. The 0.08 mm filament was presented before the 0.12 mm with the COBO. The pressure exerted by 0.08 mm and 0.12 mm filaments was calibrated using the same laboratory set-up (Table 2.3). Briefly, the subject was seated in front of the instrument and the nozzle was moved towards the centre of the cornea by controlling the joystick until it reached the working distance of 5 mm. Then the BOPM stimulus was delivered in which the type, duration and temperature was controlled by manipulating an electronic touch screen.

2.4.2.3.1 Procedure for measurements with COBO

The subject was seated at a slit lamp microscope. The COBO was mounted on the slit lamp (Figure 2.7), such that movement in the X, Y and Z planes was possible to ensure that the tip of the filament remained perpendicular to the ocular surface for each measurement. The 0.08 mm filament was used first and the filament length set at 60 mm, since this was the lowest stimulus intensity available. Subjects were advised to alternately fixate on one of two targets which allowed thresholds of corneal apex (central cornea) and the inferior conjunctiva, 2 mm vertically below a tangent to the inferior limbus (Stapleton et al 2004) as described in 2.3.2.3, between presentations of stimuli at each site. Subjects were asked to blink twice and then hold their eyes open. The filament was smoothly advanced to touch the ocular surface and was withdrawn once the investigator observed a bend in the filament. Subjects were asked to respond with ‘yes’ or ‘no’ to indicate whether they had felt the stimulus. The ascending method

of limits (Golebiowski et al 2011) was used, with four stimulus presentations made at each intensity level and threshold determined as the level where two or more positive responses to the stimulus were recorded. Stimulus intensity was increased in 5 mm steps starting at 60 mm. The filament length was recorded as threshold, which was then converted to pressure. Measurements attempted outside the intensity range of COBO are distinguished as truncated thresholds. The technique was demonstrated to the subject and sham stimuli were applied to check for false positive responses on the left untested eye prior to the experiment.

Figure 2.7 Slit lamp mounted COBO



2.4.3 **Statistical Analysis**

Pearson (parametric) and Spearman (non-parametric) Bivariate Correlation tests were used appropriately to examine the associations between threshold results using the BOPM and COBO with a 95% confidence level considered to be statistically significant.

2.4.4 **Results**

The age range of subjects (15F:3M) in the study was 21-32 (mean 25.0±4.4) years. The measurement was performed in a room with an ambient humidity range of 38-46% and a temperature 23-25ºC.Four positive responses to the lowest intensity of stimuli (at 60 mm of 0.08 mm filament) or undetectable response to the highest intensity of stimuli (at 5mm of the 0.12mm filament) were noted as truncated threshold. Table 2.2 displays the means and standard deviations of thresholds for each instrument. Since the Comparison of the Cochet-Bonnet (COBO) and the Belmonte Ocular Pain Meter (BOPM) study and the BOPM repeatability study were performed at the same time,

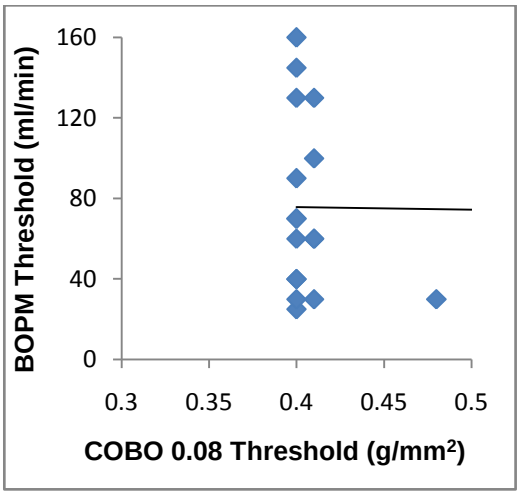
both studies obtained a single ethics approval from Human Research Ethics Advisory panel (HREA10025) with similar subject recruitment.

Table 2.2 The Means and Standard Deviations of Corneal and Inferior Conjunctival Thresholds as measured with the Belmonte Ocular Pain Meter and the Cochet-Bonnet (n =18).

Aesthesiometer Thresholds	Cochet-Bonnet (g/mm^2)		Belmonte Ocular Pain Meter (ml/min)
	0.12 mm filament	0.08 mm filament	
	(mean ± SD)		
Central cornea	0.57 ± 0	0.4 ± 0.1	75.6 ± 42.7
Inferior conjunctiva	4.1 ± 3.3	3.3 ± 5.6	84.2 ± 36.4

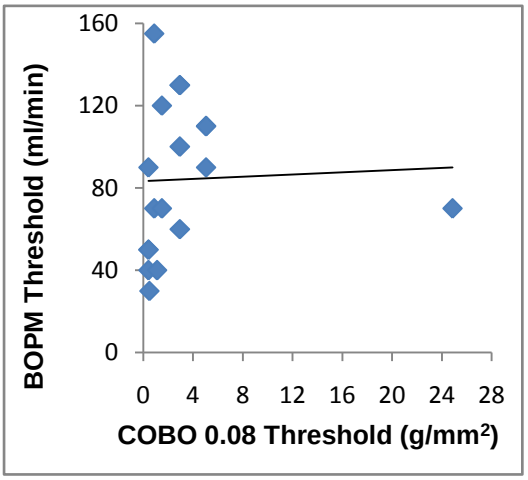
There was no significant association between measurements made with the BOPM and the COBO (0.12 mm or 0.08 mm filaments) for the cornea or the inferior conjunctiva (Figures 2.8 to 2.10). At central cornea, all COBO thresholds were truncated at the lowest stimulus intensity 0.57 g/mm^2 (0.12 mm) while 60% were truncated at 0.4 g/mm^2 (0.08 mm). At the inferior conjunctiva, 33% of the threshold were truncated at the lowest stimulus intensity 0.57 g/mm^2 (0.12 mm) while 17% were truncated at 0.4 g/mm^2 (0.08 mm). Thresholds for the two filaments were significantly associated at the inferior conjunctiva but not on the cornea (Figure 2.11). The associations remained following removal of the outlier.

Figure 2.8 Belmonte Ocular Pain Meter **(**BOPM) threshold plotted against Cochet-Bonnet (COBO) of 0.08mm filament for central cornea (n=18).



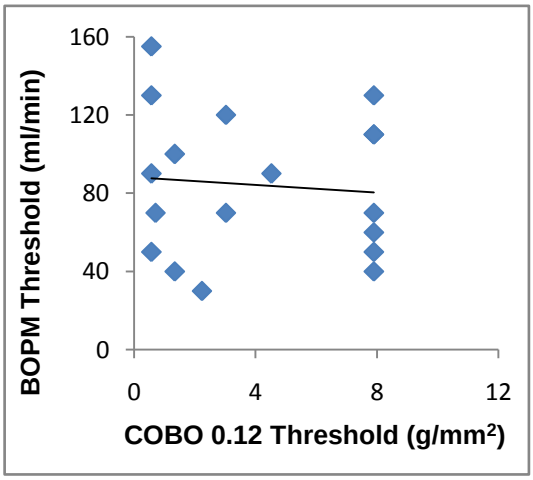
There was no significant association between measurements (r = -0.2, p = 0.05).

Figure 2.9 Belmonte Ocular Pain Meter **(**BOPM) threshold plotted against Cochet-Bonnet (COBO) of 0.08 mm diameter for the inferior conjunctiva (n=18).



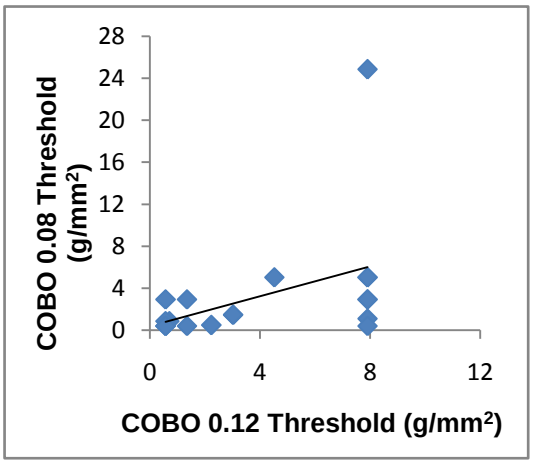
There was no significant association between measurements (r=-0.2, p = 0.95)

Figure 2.10 Belmonte Ocular Pain Meter **(**BOPM) threshold plotted against (Cochet-Bonnet (COBO) of 0.12 mm diameter for the inferior conjunctiva (n=18).



There was no significant association between measurements (r = -0.12, p = 0.63)

Figure 2.11 The threshold of thread 0.12 mm plotted against thread 0.08 mm for the inferior conjunctiva (n=18).



There was a significant association between measurements (r = 0.56, p = 0.02) which remained with the removal of the outlier.

2.4.5 **Discussion**

Due to the nature of the different stimuli, significant differences between the instruments have been observed in these subjects without ocular surface disease. The COBO aesthesiometer's nylon thread provides a purely mechanical stimulus whereas the BOPM uses a jet of air at theoretically corneal temperature to simulate a mechanical stimulus. However, the temperature of the BOPM's jet of air was found to be similar to room temperature (Lum 2013).

The COBO filament provides a static stimulus, whereas movement of the BOPM airflow over the corneal surface may additionally stimulate mechanosensory and polymodal receptors (MacIver & Tanelian 1993).The COBO stimulus is localised and temperature-neutral while stimulus of BOPM may be influenced by a change in ocular surface temperature. This is possible since some temperature sensitive "cold" nociceptors on the cornea and thermosensitive neurons in the conjunctiva are also recruited unintentionally (Murphy et al 1998). Higher corneal sensitivity has previously been shown with stimulation by airflow that is cooler than the corneal surface (Belmonte et al 1999, Stapleton et al 2004).

The tip of the COBO's nylon filament exerts pressure on a small fixed corneal area, whereas the BOPM exerts a non-uniform pressure on a larger area (Lum 2013), and the pressure decreases as distance from the airflow aperture increases. Stimulation of a larger corneal area recruits more terminal receptors within each receptive field, as well as stimulating more receptive fields, and hence more corneal neurons. Mechanical corneal threshold is understood to decrease with larger areas of stimulation (Belmonte et al 1999) and was according to Weber's law which proposed of a just noticeable difference in a stimulus is proportional to the magnitude of the original stimulus (Weber E.H 1834).

The comparison between the two filaments of the COBO demonstrated a truncation of the threshold at 60 mm in more than half of the subjects (0.08 mm) and in all subjects (0.12 mm) at the central cornea. This truncation indicated the limitation of COBO in detecting a lower threshold especially at the central cornea and may have resulted in the lack of significant association between measurements in the 0.12 mm and 0.08 mm threads. This limitation of COBO may be resolved with use of a non-contact aesthesiometer with a wider range of stimulus intensity which eliminates the threshold truncation. In contrast to the cornea, a significant association between the pressure exerted by the 0.08 mm and 0.12mm filament at the inferior conjunctiva was observed which might be due to a fewer truncated measurements on the inferior conjunctiva.

It is important to consider the differences in stimulus type delivered by different instruments and the lack of association between measurements made with the contact (COBO) and non-contact aesthesiometers when choosing a research instrument. Differences in stimulus modalities and the truncation of the sensation thresholds (in COBO) mean that the measurements using different instruments cannot be compared. These differences may have led to reporting of corneal hyposensitivity in studies using the COBO as opposed to corneal hypersensitivity in studies using non-contact aesthesiometers (CBA) in dry eye patients as described in section 1.6.

Due to the limitations of the BOPM described in section 2.2, COBO was used to measure ocular surface sensitivity (inverse of threshold) in the studies in this thesis. Therefore, it was appropriate for COBO to be calibrated and its repeatability to be determined before the studies were carried out.

2.5 Cochet-Bonnet Aesthesiometer (COBO) Calibration

2.5.1 **Aim**

To measure the pressure exerted by the 0.08 mm diameter filament of the COBO.

2.5.2 **Method**

Calibration was performed on two 0.08 mm filaments that were used throughout the studies in this thesis. The COBO was suspended vertically above a laboratory analytical microbalance (Scientech ESA80, range 0–80g, precision ±0.1 mg) to allow the filament to touch the balance plate perpendicularly, while the instrument housing case was still attached by a chuck to a height adjuster (Figure 2.12). Using the longest filament length of 60 mm, the COBO was gradually lowered with the height adjuster until the filament came into contact with the balance plate. The balance readings were noted at the point at which the first bend of the thread was observed. Five readings were recorded from each scale (5 mm) of the filament. The diameter of the filament was measured with a Nikon V-24B profile projector (Nikon Corporation, Japan) (Figure 2.13). The measured force values were then converted to pressure (g/mm^2) by dividing the average force of the filament (g), obtained from the above procedure, by the area stimulated by the tip of the filament at the values of 0.0064 mm^2 for filament 1 and 0.0057 mm^2 for filament 2.

Figure 2.12 Nikon V-24B Profile projector

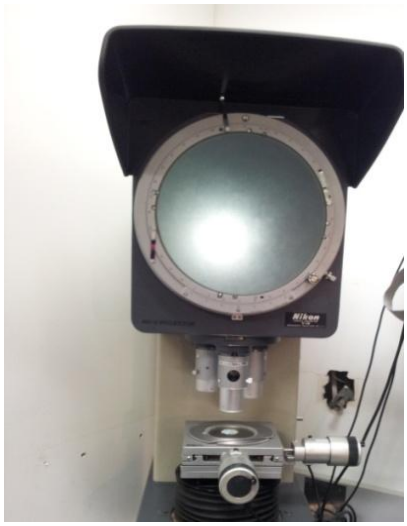
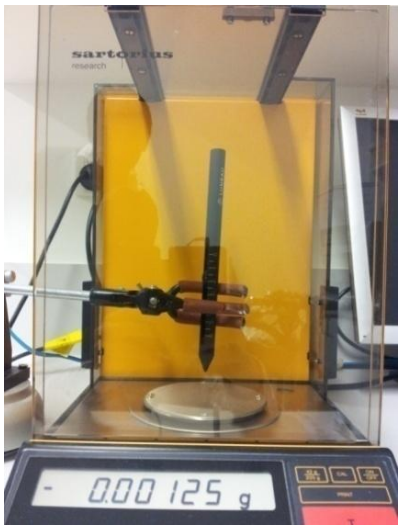


Figure 2.13 Height adjuster and Laboratory analytical balance



2.5.3 **Statistical Analysis**

Data normality was assessed using the Shapiro-Wilk test (p> 0.05). Differences in pressure of the 0.08 mm filaments were evaluated using the Mann-Whitney test (p<0.05).

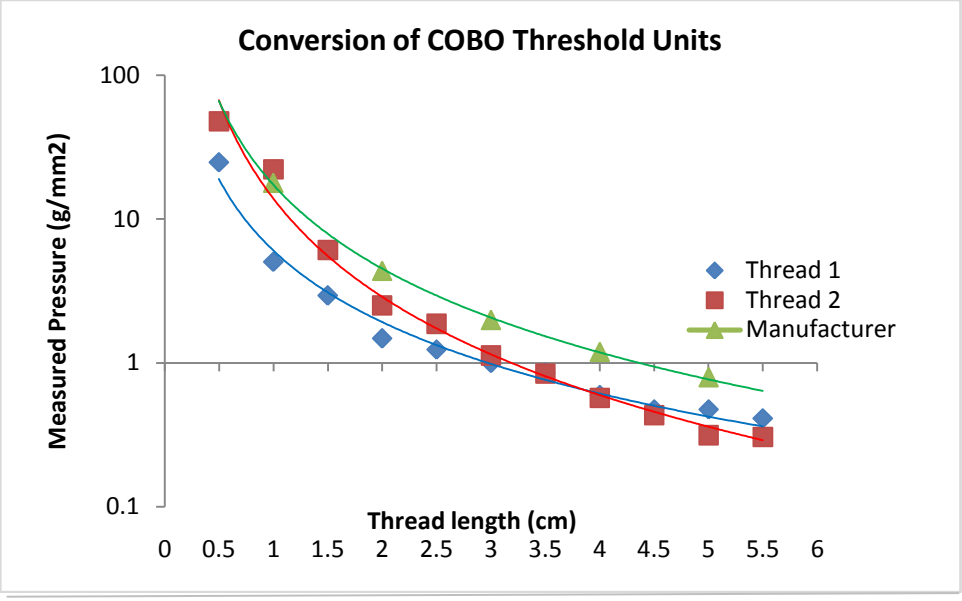
2.5.4 **Results**

As expected, the pressure was not normally distributed with p<0.001 for filament 1 and p = 0.01 for filament 2. There was no significant difference in pressure between the two 0.08 mm filaments (p = 0.82). Table 2.3 displays the converted pressure units from cm to g/mm^2, standard deviations in the two 0.08 mm filaments and pressure provided by the manufacturer. The measured pressure was consistently lower than the manufacturers' reported pressures.

Table 2.3 Conversion table of measurements from length to pressure forfilaments1 and 2 (both 0.08 mm) and 0.12 mm filament calibrated by Chao (2013) and threshold values provided by the manufacturer.

Length (cm)& Pressure ± SD (g/mm^2)	**6cm**	**5.5cm**	**5.0cm**	**4.5cm**	**4cm**	**3.5cm**	**3cm**	**2.5cm**	**2cm**	**1.5cm**	**1cm**	**0.5cm**
Filament 1 (0.08 mm)	0.40± 0.02	0.41± 0.04	0.47± 0.05	0.48± 0.08	0.61± 0.06	0.86± 0.11	1.10± 0.06	1.24± 0.12	1.48± 0.22	2.94± 0.33	5.04± 1.20	24.83± 11.23
Filament 2 (0.08 mm)	0.11± 0.01	0.30± 0.02	0.31± 0.03	0.43± 0.02	0.57± 0.07	0.84± 0.06	1.13± 0.08	1.87± 0.09	2.50± 0.12	3.82± 0.37	5.50± 0.91	19.10 ± 3.80
Manufacturer's filament (0.08 mm)	0.41	-	0.81	-	1.19	-	1.99	-	4.38	-	17.90	-
0.12 mm (C.Chao)	0.57	0.70	0.86	1.09	1.34	1.74	2.23	3.02	4.52	7.90	15.24	56.22

Figure 2.14 COBO filament length (cm) as a function of pressure after semi-log transformation



2.5.5 **Discussion**

The conversion table provided by the COBO manufacturer allows the threshold of the filament in cm to be converted to pressure exerted on the ocular surface in g/mm^2. However, as presented in Figure 2.14, there is a difference between the measured pressure of the filaments and the manufacturer's, for the same filament length. Furthermore, there was a sudden sharp rise of pressure at 0.5 and 1.0 cm as previously demonstrated (Golebiowski et al 2011).Inconsistent pressure was exerted by nylon even with equal diameter and length (Lawrenson & Ruskell 1993, Norn 1973) which could be due to the environmental effects such as humidity and wear (Millodot 1967, Murphy et al 1998). Therefore, calibration of COBO thread prior to any study undertaken is recommended to ensure a more accurate ocular surface threshold measurement.

2.6 Repeatability of Cochet-Bonnet (COBO)

2.6.1 **Aim**

To determine the repeatability of the COBO

2.6.2 **Method**

2.6.2.1 Study Design

Twenty nine healthy subjects were recruited for the measurement of the Cochet-Bonnet aesthesiometer repeatability. The study was performed as part of a randomised double masked placebo-controlled intervention study investigating the effect of fish oil on ocular comfort where the study measurements were repeated after three months. Therefore, ocular surface thresholds were measured with COBO twice, three months apart and the data were reported for the placebo group.

The interventional study also involved venous blood collection which requires for the exclusion of subjects with infectious disease transmittable by blood e.g. HIV/AIDs as listed in the exclusion criteria. However, only the ocular surface sensitivity measurements were analysed in this repeatability study.

Thresholds of the central cornea and the inferior conjunctiva of the right eye were measured as described in Section 2.5.2.2.1, using filament 1 for half of the subjects and filament 2 to replace the noticeable bended filament 1, for the remaining subjects [filaments 1 and 2 were described in detail in section 2.6. and the respective thresholds were used accordingly (Table 2.3)]. Measurements were conducted at the same time at each visit between 8 A.M and 7 P.M to mitigate against the effects of diurnal fluctuations.

2.6.2.2 Subjects

A convenience sample of 29 subjects was recruited. Convenience sample means the subjects were not purposely enrolled for the repeatability study only but instead participated as controls in another interventional study and the baseline results were utilised herein. Based on this sample size, the study has the power of 80% and alpha = 0.05 to detect a difference of 1.3 (g/mm^2) of corneal threshold between the repeated measurements. Subjects were recruited from the School of Optometry and Vision Science, University of New South Wales (UNSW) via email and from the UNSW campus and general community via a flyer. Ethics approval was obtained from the Human Research Ethics Committee (HREC 10110) of the University of New South

Wales, Sydney, Australia and followed the tenets of the Declaration of Helsinki. Signed informed consent was obtained from each participant prior to enrolment in the study (Appendix 2).

Inclusion criteria

At least 18 years old,

Exclusion criteria

- A prior diagnosis of moderate or severe dry eye disease by a medical or ophthalmic practitioner,
- Any systemic disease that would preclude participants from safely ingesting dietary supplementation with combination omega oils,
- Use of any polyunsaturated fatty acid-containing dietary supplements (such as fish oil, evening primrose oil, linseed oil) up to 12 weeks prior to start of the study,
- Use of any anticoagulant or blood thinning medications (such as Heparin, Warfarin or Aspirin) up to 12 weeks prior to start of the study,
- Use of any of the following medications (including steroids) up to 12 weeks prior to start of the study:

: Topical or systemic ocular medication, category S3 and above. Schedule 3 (S3) drugs and poisons are also known as Pharmacist Only Medicines, are substances and preparations for therapeutic use that: are substantially safe in use but require professional advice or counselling by a pharmacist, professional medical or dental, management or monitoring.

- Use of systemic or topical medications that affect ocular physiology e.g. anti-acne medications such as Roaccutane and corticosteroid or immunosuppressant medications such as Hydrocortisone, Prednisolone and antihistamine medications such as Claritine

- Any systemic disease that would affect ocular health e.g. Graves' disease, and auto-immune diseases such as ankylosing spondylitis, multiple sclerosis and systemic lupus erythematosis
- Any infectious diseases transmittable by blood e.g. HIV/AIDs, Hepatitis
- Eye surgery within 6 months immediately prior to enrolment for this study
- Previous corneal refractive surgery, and
- Pregnancy or breastfeeding.

2.6.3 **Statistical Analysis**

Data normality was assessed using the Shapiro-Wilk test (p>0.05). Differences between visits were evaluated using the Wilcoxon Signed-rank test and statistical significance was set at p<0.05. Bland and Altman graphs were plotted, and the CoR, LoA and bias were calculated (Bland & Altman 1986) as described in section 2.2.3.

2.6.4 **Results**

Normal to mild dry eye males, menstruating and postmenopausal women (20F:9M) within the age range of 19 - 76 years (mean 38 ± 14) were recruited and 11 were regular contact lens wearers. Measurements were carried out in a room with an ambient humidity range of 35-72% and a temperature 19 -25ºC. Threshold results for the two visits were not normally distributed (Table 2.4).There was no statistically significant difference between the two measurements (visit 1 and visit 2) at either location. Bland and Altman plots indicate the LoA and bias values for all central cornea and inferior conjunctival thresholds (Figures 2.15 and 2.16) and their truncated thresholds (Figures 2.18 and 2.19) respectively.

Table 2.4 The Median and Inter Quarter Range of Corneal and Inferior Conjunctival Thresholds by Visit (3 months apart) and Location (n= 29)

	Central Corneal Threshold Median (Inter Quarter Range) (g/mm^2)		Inferior Conjunctival Threshold Median (Inter Quarter Range) (g/mm^2)	
	All Subjects	**Non-truncated**	**All Subjects**	**Non-truncated**
Visit 1	0.40 (0.40-0.55)	0.51 (0.41-0.86)	3.74 (0.49-19.66)	1.24 (0.47-5.04)
Visit 2 (After 3 months)	0.40 (0.35-0.42)	0.40 (0.35-0.61)	1.24 (0.42-19.66)	0.80 (0.42-19.66)
Wilcoxon-Signed rank test *p* value	0.07	0.56	0.72	0.79
± Coefficient of Repeatability (CoR) g/mm^2	0.5	0.6	22.4	22.33

There was no statistically significant difference between the two measurements (visit 1 and visit 2) at either location (p=0.04).

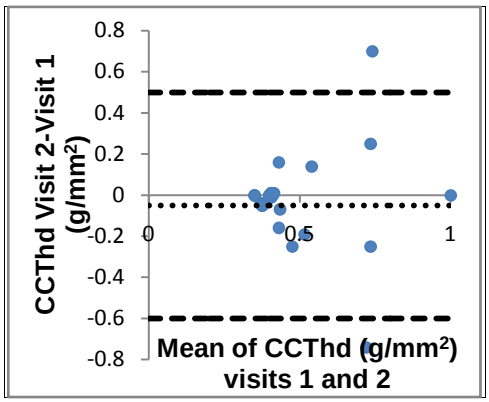
Median corneal thresholds on the two visits were not significantly different (Table 2.4). The bias between the two visits was 0.05 g/mm^2 and the CoR of ±0.5 g/mm^2 indicates that 95% of the differences between the two repeats can be expected to lie between +0.57 and -0.47g/mm^2 (Figure 2.15).

Median conjunctival thresholds on the two visits were not significantly different (Table 2.4). The bias between the two visits was 0.4 g/mm^2 and the CoR of ±22.4 g/mm^2 indicates that 95% of the differences between the two repeats can be expected to lie between +22.8 and -22.0 g/mm^2 (Figure 2.16).

Fourteen of 29 corneal measurements were truncated at least in one visit. Median corneal thresholds on the two visits were not significantly different (Table 2.4). The bias between the two visits was -0.02 g/mm^2 and the CoR of ± 0.6 g/mm^2 indicates that 95% of the differences between the two visits can be expected to lie between +0.6 and -0.6g/mm^2 (Figure 2.17).

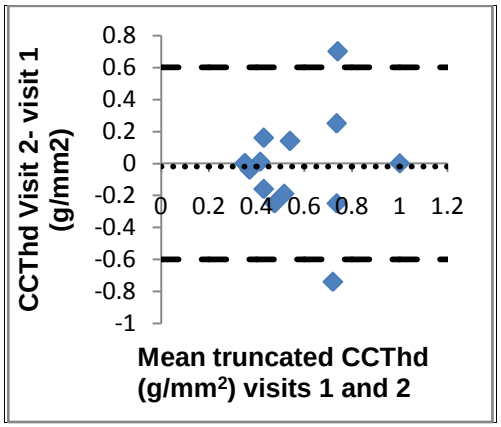
Five of 29 conjunctival measurements were truncated at least in one visit at the highest stimulus intensity. Median conjunctival thresholds on the two visits were not significantly different (Table 2.4).The bias between the two visits of corneal threshold was -0.80 g/mm^2 and the CoR of ± 22.3 g/mm^2 indicates that 95% of the differences between the two repeats can be expected to lie between +21.5 and -23.1 g/mm^2 (Figure 2.18).

Figure 2.15 Difference between thresholds measurements of the first and second visits was plotted against their means for the central corneal threshold (CCThd) (n=29). The dotted line represents a bias of -0.05 g/mm^2.The dashed lines represent the limits of agreement of +0.5 and –0.6 g/mm^2.



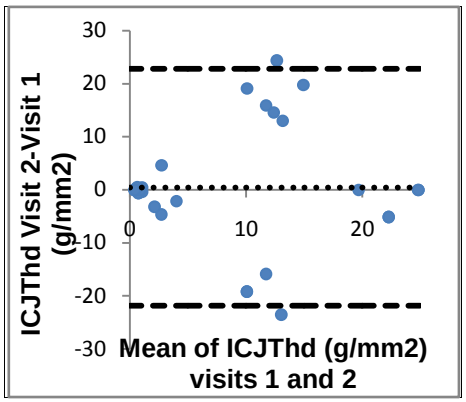
There was no significant difference of the thresholds measurements between the first and second visits (p=0.07) as displayed in table 2.4.

Figure 2.16 Difference between thresholds measurements of the first and final visits was plotted against their means for the central corneal threshold (CCThd) (n=29) without truncated thresholds. The dotted line represents a bias of -0.02 g/mm^2.The dashed lines represent the limits of agreement of +0.6 and –0.6g/mm^2.



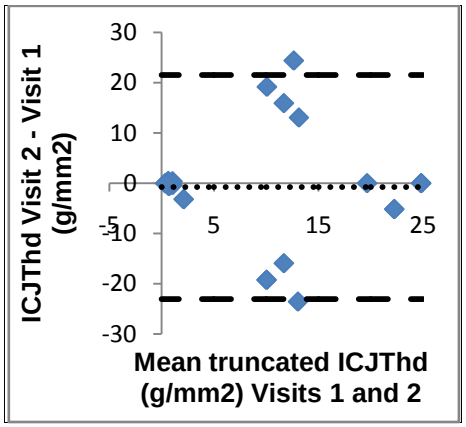
There was no significant difference of the thresholds measurements between the first and second visits (p=0.56) as displayed in table 2.4.

Figure 2.17: Difference between thresholds measurements of the first and final visits was plotted against their means for the inferior conjunctival threshold (ICJThd) (n=29). The dotted line represents a bias of 0.4 g/mm^2.The dashed lines represent the limits of agreement of +22.8 and -21.9 g/mm^2.



There was no significant difference of the thresholds measurements between the first and second visits (p=0.72) as displayed in table 2.4.

Figure 2.18 Difference between thresholds measurements of the first and final visits was plotted against their means for the inferior conjunctival threshold (ICJTh) (n=29) without truncated thresholds. The dotted line represents a bias of -0.8 g/mm^2.The dashed lines represent the limits of agreement of +21.5 and –23.1 g/mm^2.



There was no significant difference of the thresholds measurements between the first and second visits (p=0.79) as displayed in table 2.4.

2.6.5 **Discussion**

There was no significant difference between the thresholds of cornea in these normal to mild dry eye males, menstruating and postmenopausal women from the two visits which was supported by the narrow limits of agreement (magnitude 1.1 g/mm2) and the bias of almost 0 (0.05 g/mm^2). However, half of the corneal thresholds were truncated in this study as previously demonstrated (Golebiowski et al 2011, Murphy et al 1998). Nevertheless, COBO was still repeatable even with the truncated measurements excluded.

In contrast, a wider agreement (magnitude of 44.7 g/mm^2) and a bias of 0.4 g/mm^{2}for inferior conjunctiva measurements suggest that the COBO has a lower repeatability when used at the inferior conjunctiva than the cornea. This finding is consistent with the greater variance in conjunctival sensitivity than in cornea as measured with the COBO (Situ et al 2010). In addition, regions of the conjunctiva were identified to be less sensitive than the central cornea with COBO (Norn 1973). Although the conjunctiva contains numerous structurally specialised corpuscular nerve endings (Lawrenson & Ruskell 1993), it has fewer free nerve endings than the cornea (Ruskell 1985), which might contribute to a higher threshold (lower sensitivity) and lack of similar responses obtained at the conjunctiva from the subjects on both visits.

Size effect was the other important factor affecting the repeatability of the instrument as demonstrated from the Bland and Altman plots. Based on Figure 2.16, the CoRs calculated may underestimate the repeatability of corneal thresholds below approximately 0.6 g/mm^2 and overestimate repeatability of thresholds above approximately 0.6 g/mm^2. CoR was smaller and calculated to be (±0.16 g/mm^2) when only thresholds below 0.6 g/mm^2 were considered, which indicates that the more repeatable corneal thresholds is at the filament lengths of 40 mm (less than 0.6g/mm^2). Therefore, it is suggested that the 0.08 mm filament is switched to 60 mm of the 0.12 mm filament at this point when 40 mm for the 0.08 mm cannot be detected during threshold measurement at the cornea. In order to overcome the less repeatable measurements at low sensitivity (more than 0.6 g/mm^2), utilising only the longer filament lengths of both filaments may be useful as the relatively smaller interval is at the longer filament length of the 0.12 mm than lengths less than 40 mm of the 0.08 mm

filament. In the inferior conjunctiva, the CoR was not smaller as when only thresholds below 5.5 g/mm^2 (10mm) were considered, which suggests that COBO maintains a low repeatability even with the removal of the truncated threshold. This requires confirmation in a larger study which includes a greater number of subjects with higher thresholds.

Only a limited number of studies of ocular surface sensitivity measurement have reported repeatability data. The CoR for the CRCERT-Belmonte aesthesiometer has been shown to range between 18.3-37.3 ml/min for central corneal measurements, which is approximately one quarter to one half of normal corneal threshold values for that instrument (Golebiowski et al 2005, Stapleton et al 2004). In comparison, the CoR for the COBO aesthesiometer was ±0.52 g/mm^2, which approximates one third of the normal corneal threshold (2.7 ± 0.5) g/mm^2 (Jalbert et al 2012). The repeatability of the COBO is therefore reasonable, although the mechanical thresholds reported in published studies are not easy to compare to the current study since the different types of aesthesiometers used are likely to stimulate different corneal nerve endings (Aδ versus C nerve fibres respectively) (Chao et al 2014, Darwish et al 2007). In addition, the CoR for the COBO is influenced by the non-linear interval of the measurement range.

2.7 Conclusion

Despite the truncation of thresholds with the COBO at the low end of the stimulus range, its repeatability on the cornea is accepted. However, the repeatability of conjunctival threshold using this instrument is poor. Although the Cochet-Bonnet aesthesiometer is a repeatable tool to measure corneal threshold, care should be taken in the measurement of both subjects with high levels of sensitivity (due to truncation of stimulus intensity) and with low levels of sensitivity (due to lesser repeatability). In addition, the calibration of the COBO's filament is still required prior to use since there is a difference between the pressures exerted by the filaments of the same filament length.

Although non-contact aesthesiometers may be preferable for measuring threshold where the corneal epithelium is damaged or fragile, such as recurrent corneal erosion and post-keratoplasty cases, repeatability tests on such instruments are highly

recommended. Threshold measurements using contact and non-contact aesthesiometers cannot be easily compared due to stimulus differences.

CHAPTER 3

The Effects of Circulating Sex Hormone Levels on Ocular Surface Sensitivity and Dry Eye Symptoms and Signs in a Normal to Mild Dry Eye population

3.1 Introduction

As symptoms and signs of dry eye are consistently reported more frequently in females than males (Chew et al 1993, Cho & Yap 1993, Farris et al 1986, Guillon & Maïssa 2010, Lamberts et al 1979, Moss et al 2000, Sakamoto et al 1993), this suggests a potential role for sex hormones in the pathophysiology of dry eye.

Changes to physiological levels of oestrogen, androgen or progesterone may affect ocular symptoms (Erdem et al 2007, Gagliano et al 2014, Mamalis et al 1996, Scuderi et al 2012), ocular surface sensitivity (Millodot & Lamont 1974, Riss et al 1982), tear function (Forsblad-d'Elia et al 2009, Marcozzi et al 2003, Mathers et al 1998, Sullivan et al 2003, Versura et al 2007) and meibomian gland function (Krenzer et al 2000, Sahin & Kartal 2011). For instance, a lower level of circulating testosterone is associated with dry eye in women (Mamalis et al 1996) while a higher level of circulating oestradiol may exacerbate the symptoms of ocular dryness and foreign body sensation in postmenopausal women using hormone replacement therapy (HRT) (Erdem et al 2007). Nevertheless, several other clinical studies were unable to demonstrate changes in symptoms or tear function with the use of HRT (Kuscu et al 2003, Piwkumsribonruang et al 2010, Taner et al 2004). To date, there is no report on the effects of the circulating sex hormone levels on dry eye symptoms and signs in a normal non postmenopausal population.

Age is a risk factor for dry eye in both males and females (DEWS 2007)(Smith et al 2007) which may be related to the alteration in sex hormone levels with age (Lamberts et al 1997). Several epidemiological studies showed increasing dry eye symptoms with age(Lee et al 2002, Lin et al 2003, McCarty et al 1998, Moss et al 2008) except in one study (Schein et al 1997b). Aging affects tear function (Hagele et al 1994, Lamberts et

al 1979, Mathers et al 1996, Paschides et al 1991, Patel & Farrell 1989, Sakamoto et al 1993, Versura et al 2006), meibomian gland morphology (Bron et al 1991, Hykin & Bron 1992, Yamaguchi et al 2006) and function (Arita et al 2008, Nien et al 2011, Norn 1987). One study demonstrated that age was a better predictor of dry eye than total testosterone, prolactin and follicle stimulating hormone levels in a sample of 110 women aged 35 to 60 years (Mathers et al 2002).

Both age and intrinsic aspects of gender, such as immune system regulation, cyclic variations in hormone levels, relative hormone levels and psychosocial factors may confound the relationship between hormone levels, and symptoms and signs of dry eye. Significant associations between ocular surface sensitivity and either age or gender were previously demonstrated (Acosta et al 2006, Bourcier et al 2005, Golebiowski et al 2008, Millodot 1977a, Situ et al 2008b). However, ocular surface staining was not associated with age and gender in a large dry eye epidemiological study (McCarty et al 1998). There were also no gender differences in clinical signs such as non-invasive break-up time (NIBUT) (Ozdemir & Temizdemir 2009) and meibomian gland assessment score (Schaumberg et al 2011, Viso et al 2012) except for a higher prevalence of meibomian gland disease in men (Siak et al 2012). Given these equivocal findings, it is important to understand the impact of both age and gender on the relationship between dry eye and sex hormones in a normal population.

While symptoms of dry eye are associated with age and gender, other possible confounders include contact lens wear. Ocular dryness and discomfort are frequently reported by contact lens wearers, and the symptoms worsen toward the end of the day (Begley et al 2000). In addition, contact lens wearers are five times more likely to report dry eye than spectacle wearers (Nichols et al 2005).

Although the combination of progesterone and oestrogen has previously improved the dry eye symptoms and signs (Affinito et al 2003, Altintaş et al 2004, Coksuer et al 2011, Guaschino et al 2003, Jung et al 2010, Kuscu et al 2003, Uncu et al 2006), there was no investigation of the effect of progesterone alone on dry eye. This study aimed to establish associations between oestrogen, androgens, and progesterone levels, and dry eye symptoms and signs in a sample of normal subjects. The study also aimed to identify the potential predictors of symptoms from a panel of variables which included sex hormone levels, ocular surface sensitivity, dry eye clinical signs, age and contact lens wear by gender and hormone status.

3.2 Aims

3.2.1 **Primary Aims**

a) To investigate the associations between circulating levels of oestradiol, total testosterone, free testosterone, progesterone, dehydroepiandrosterone sulfate (DHEA-S), sex hormone binding globulin (SHBG), 5 alpha-androstane-3 alpha 17 beta-diolglucuronide (3α-diol G), the ratios between oestrogen and androgens and ocular surface sensitivity; and dry eye symptoms and signs in a normal-to-mild dry eye population of both genders, ‘all subjects’, then in ‘males only’ and ‘females only’.

b) To identify the predictors for ocular symptoms from a panel of variables which include plasma sex hormone concentrations, the ratios between oestrogen and androgens, ocular surface sensitivity, clinical signs, age and contact lens wear in a normal-to-mild dry eye population of both genders ‘all subjects’, and in ‘females only’.

3.2.2 **Secondary Aim**

a) To examine the effect of gender on ocular symptoms, ocular surface sensitivity and clinical signs of dry eye in a normal-to-mild dry eye population.

3.3 Hypotheses

a) A higher circulating level of oestradiol and the ratios between oestrogen and androgens are associated with

    i. higher symptoms

    ii. lower ocular surface sensitivity

    iii. greater signs of dry eye

b) A higher circulating level of testosterone, free testosterone, 3α-diol G and DHEA-S is associated with

    i. lower symptoms

    ii. higher ocular surface sensitivity

    iii. fewer dry eye signs

c) Age is associated with higher symptoms, lower ocular surface sensitivity and greater dry eye signs.

d) Females display higher scores of ocular symptoms, lower ocular surface sensitivity and greater dry eye signs compared to males.

3.4 Method

This cross-sectional single visit study was conducted at the School of Optometry and Vision Science (SOVS). Subjects were enrolled for a two-hour visit between 8 A.M and 8 P.M. Ethics approval was obtained from the Human Research Ethics Committee (HREC 10110) of the University of New South Wales, Sydney, Australia and followed the tenets of the Declaration of Helsinki. Signed informed consent was obtained from each participant prior to enrolment in the study (Appendix 2).

3.4.1 Subjects

A convenience sample of 76 subjects within the age range between19-76 (54F:22M) was recruited. Convenience sample means the subjects were not purposely enrolled for this preliminary study but they instead participated in an interventional study and the

baseline results were utilised herein. Based on this sample size, the study has the power of 85% and alpha = 0.05 to demonstrate an association between circulating oestradiol and dryness sensation of the Numerical Ratings Questionnaires (NRS) with a rho value of -0.31. Study subjects were recruited via advertisements in community newspapers and on notice boards; generic emails circulated within SOVS staff and students; written invitations to the patients of the SOVS eye clinic; and posters and flyers placed around the campus of the University of New South Wales.

This study was performed in a normal to mild dry eye subjects.

Inclusion criteria

- At least 18 years old

Exclusion criteria

- A prior diagnosis of moderate or severe dry eye disease by a medical or ophthalmic practitioner,
- Any systemic disease that would preclude participants from safely ingesting dietary supplementation with combination omega oils,
- Use of any polyunsaturated fatty acid-containing dietary supplements (such as fish oil, evening primrose oil, linseed oil) up to 12 weeks prior to start of the study,
- Use of any anticoagulant or blood thinning medications (such as Heparin, Warfarin or Aspirin) up to 12 weeks prior to start of the study,
- Use of any of the following medications (including steroids) up to 12 weeks prior to start of the study:

: Topical or systemic ocular medication, category S3 and above. Schedule 3 (S3) drugs and poisons are also known as Pharmacist Only Medicines, are substances and preparations for therapeutic use that: are substantially safe in use but require professional advice or counselling by a pharmacist, professional medical or dental, management or monitoring.

- Use of systemic or topical medications that affect ocular physiology e.g. anti-acne medications such as Roaccutane and corticosteroid or immunosuppressant medications such as Hydrocortisone, Prednisolone and antihistamine medications such as Claritine
- Any systemic disease that would affect ocular health e.g. Graves' disease, and auto-immune diseases such as ankylosing spondylitis, multiple sclerosis and systemic lupus erythematosis
- Any infectious diseases transmittable by blood e.g. HIV/AIDs, Hepatitis
- Eye surgery within 6 months immediately prior to enrolment for this study
- Previous corneal refractive surgery, and
- Pregnancy or breastfeeding.

Only baseline data were used for the current study.

3.4.2 **Procedures**

Variables were measured in the order described below

3.4.2.1 Ocular Symptoms

3.4.2.1.1 Ocular Comfort Questionnaires

Self-administered ocular comfort questionnaires were presented to study subjects in electronic format as described below.

1. Women's Health Study (Schaumberg et al 2001) (Appendix A)

This dry eye classification questionnaire consists of three questions on the frequency of symptoms of dryness and irritation, on a 1-4 scale where 4 represents constant, 3 represents often, 2 represents sometimes and 1 represents never. The questionnaire also includes an item on previous history of clinically diagnosed dry eye. Subjects with responses of 'constant' and 'often' to dryness and irritation, or who had been previously diagnosed with dry eye were classified as having dry eye.

2. Ocular Comfort Index (OCI)(Johnson & Murphy 2007) (Appendix B)

The12-item OCI questionnaire, addressed six symptoms: dryness, grittiness, stinging, tiredness, pain and itchiness. The frequency and intensity of each symptom was explored in turn. The scores for each were entered into the OCI calculator:(http://www.iovs.org/cgi/content/full/48/10/4451/DC1)(Johnson & Murphy 2007), which gave a total score on a 0-100 scale where 100 represents the greatest discomfort.

3. Ocular Surface Disease Index [(OSDI) Allergan Inc, Irvine, California USA 2004] (Appendix C)

The 12-item OSDI questionnaire is based on a five-category Likert design, with three subscales that sequentially explored symptoms of ocular irritation, impact on vision-related functioning and environmental triggers of dry eye. The formula below was used to give a score on a 0-100 scale where 100 represent the greatest discomfort as described in [(OSDI) Allergan Inc, Irvine, California USA 2004].

OSDI = [(sum of scores) x 25]/(number of questions answered)

4. Dry Eye Questionnaire [(DEQ) Indiana University 2002, (Begley et al 2002a)] and DEQ 5 (Chalmers et al 2010) (Appendix D and E)

The frequency, intensity (assessed in the morning and afternoon) and "bothersome-ness" of discomfort; dryness; grittiness and scratchiness; burning and stinging; tiredness; and changeable and blurry vision were recorded and converted to percentages. The sum of the three "symptoms scales" was recorded as the total score from 0 to 100 where 100 represents the greatest discomfort.

In the DEQ 5, total scores of the intensity of watery eyes; both intensity and frequency of ocular comfort and dryness were added up (from the scores of the same questions of DEQ), giving a score on a 0 to 22 scale where 22 represents the greatest discomfort (Chalmers et al 2010).

5. Numerical Ratings Questionnaire (NRS) (Appendix F)

Symptoms of comfort, dryness, foreign body sensation, wateriness and burning were rated by subjects from 100 to 0 where 1 represents greatest discomfort.

6. Subjective evaluation of symptom of dryness (SESOD)(Simmons et al 2003) (Appendix G)

Symptom of dryness was recorded on a scale from 0 to 4 where 4 represents greatest discomfort.

3.4.2.2 Clinical Signs of Dry Eye

Assessments of tear function; ocular surface sensitivity and integrity and meibomian gland assessments were then carried out consecutively. The tear function and ocular surface integrity assessments were performed bilaterally with the right eye tested first while the meibomian gland assessments were performed only on the lower lid of the right eye. Tear function tests were conducted with the lens in place for contact lens wearers. A two to five minute interval was allowed between each complete assessment on both eyes to minimize reflex tearing and ocular surface changes as a consequence of the testing protocol (Akramian et al 1998).The ocular surface sensitivity was

measured only on the right eye. If the subject was a contact lens wearer, the lenses were removed for a minimum of 15 minutes prior to the procedure (Murphy et al 2001).

3.4.2.2.1 Tear Function Assessments

3.4.2.2.1.1 Tear Osmolarity

Osmolarity was measured using the Ocusense TearLab Osmolarity System (TearLab corporation, CA, US). The subject was instructed to blink normally, tilt the head towards the eye being measured and look up. The Tearlab Pen was gently touched to the tear meniscus at the lateral canthus, then replaced into its slot in the reader and the osmolarity measurement was displayed in mOsmo/L. The higher of the measurements from the two eyes was used for analysis(Lemp et al 2011).

3.4.2.2.1.2 Non invasive Tear Break-up Time (NIBUT)

NIBUT was measured using the slit lamp biomicroscope (Topcon, SL-D7, Tokyo, Japan) at 16x magnification and the handheld Keeler Tearscope-plus^{R} (Keeler, Windsor, UK). The tearscope was positioned against the subject's cheekbone and brow. The subject was instructed to blink twice and to hold the blink for as long as possible while the examiner observed the tear film. The time to first break-up in the tear film was recorded in seconds. The test was performed twice on each eye and averaged. The average score of the right and left eyes was used for analysis.

3.4.2.2.1.3 Tear Volume

Tear volume was based on the wet length of the phenol red thread (PRT ZONE-QUICK, Showa Yakuhin Kako Co., Ltd, Japan). The thread was placed in the lower fornix for 15 seconds and the length of the colour indicator was recorded in mm upon removal. The subject was instructed to blink normally before and throughout the test while looking ahead. The average length of the right and left eyes was used for analysis.

3.4.2.3 Ocular Surface Sensitivity

3.4.2.3.1 Cochet-Bonnet Aesthesiometer (Luneau Ophthalmologie, France)

Ocular surface sensitivity measurement has been described in 2.2.1 (Methods development chapter). Measurements were performed at the corneal apex and lower inferior conjunctiva of the right eye only using the ascending method of limit (Golebiowski et al 2011).

3.4.2.3.2 Ocular Surface Integrity Assessments

3.4.2.3.2.1 Corneal Staining

Twenty µl of a solution made by dipping a sodium fluorescein strip (1 mg, Fluorets, Bausch and Lomb, Australia) in 200 µl of normal saline (sodium chloride injection BP 0.9%, 45 mg in 5 mL, Pfizer Pty Limited, Australia) for one minute (Delaveris et al 2011), was dispensed into the lower fornix. The cornea was assessed with a slit lamp at 16x magnification through Wratten filter number 12 under cobalt blue illumination. The subject was instructed to blink normally to spread the dye uniformly over the ocular surface. Corneal staining was graded with a single overall score according to the modified Oxford grading scale (0 to 5, 0.5 steps)(Bron et al 2003)(Appendix I). The average score of the right and left eyes was used for analysis.

3.4.2.3.2.2 Conjunctival Staining

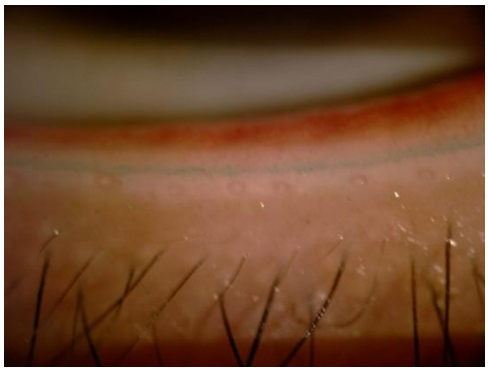
Twenty µl of a solution made by dipping a Lissamine green strip (1.5 mg OpGreen, Ophtechnics unlimited, India) in 200 µl of normal saline (sodium chloride injection BP 0.9%, 45 mg in 5 mL, Pfizer Pty Limited, Australia) for one minute (Delaveris et al 2011), was dispensed into the lower fornix. The conjunctiva was assessed with a slit lamp at 16x magnification under white light. The subject was instructed to blink normally to spread the dye uniformly over the ocular surface. Conjunctival staining was graded for nasal, temporal and inferior quadrants separately according to the modified Oxford grading scale (0 to 5, 0.5 steps)(Bron et al 2003) (Appendix I). The final score for conjunctival staining was the total score of the three quadrants and therefore ranged from 0 to 15 (0.5 steps). The average total score of the right and left eyes was used for analysis.

3.4.2.3.3 Meibomian Gland, Lid Margin and Tarsal Conjunctiva Assessments

3.4.2.3.3.1 Meibomian Gland Orifice Morphology

Meibomian gland assessment was performed on the lower lid of the right eye. The lower lid was gently pressed between the thumb and index finger to shape the lid into a flat surface, allowing better access to the orifices. The view of the middle third of the lid margin was magnified by 40x with a slit lamp attached camera and an image captured. The number of orifices was then counted from the resulting image (Figure 3.1). The gland orifices were examined under 16x magnification for any abnormality such as capping, scarring, pouting and narrowing using a scale of 0 or 1 where zero is normal (Appendix J).

Figure 3.1 Magnified (40x) image of Middle Third of Lower Lid Margin



3.4.2.3.4 Meibomian Gland Secretion

Firm digital pressure was applied on the central part of lower lid margin of the right eye with the index finger (Bron et al 1991, Foulks & Bron 2003, Mathers et al 1991, Pflugfelder et al 1998, Tomlinson et al 2011). Meibomian glands located within that part were examined under 16x magnification for the number of patent glands (number of secreting glands) and meibomian gland expressibility [secretion quality and expression of the glands (effort to express)] on a scale where readings from 4 to 6 represent abnormal secretion(Appendix J).

3.4.2.3.5 Lid Margin Physiological Features

The lower lid margin of the right eye was examined under 16x magnification for any abnormality such as notching, rounding, hyperkeratinisation, foam, vascularity and telangiectasia and scored 0 or 1 where zero is normal (Appendix I).

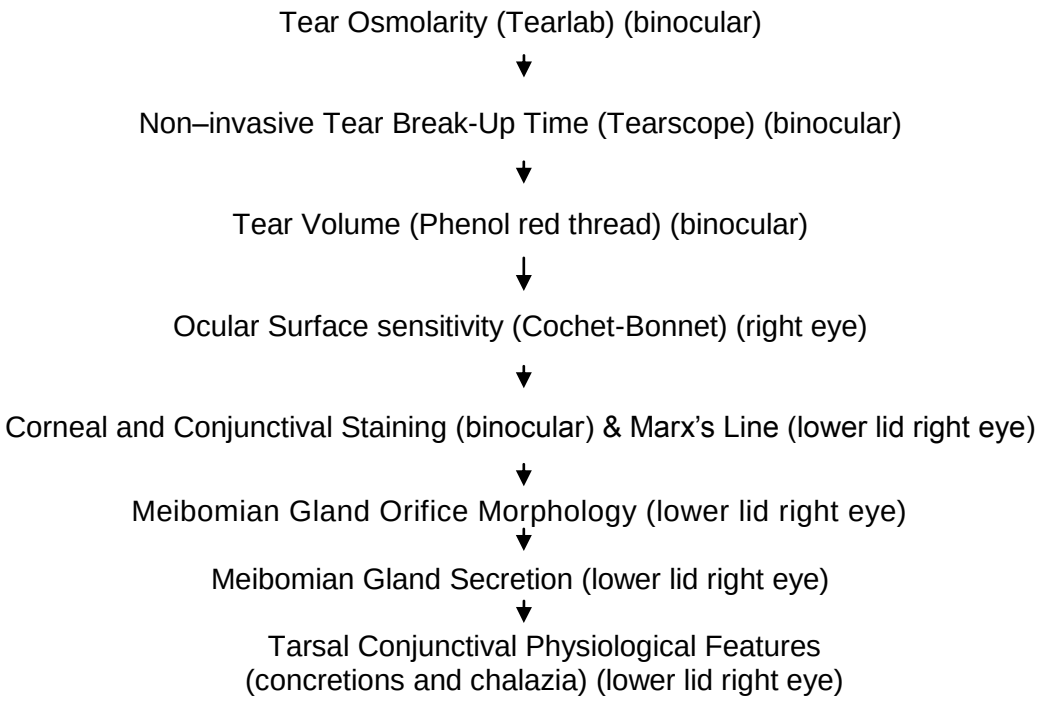
3.4.2.3.6 Marx's Line

Lissamine green staining of the lid allows visualisation and grading of Marx's line displacement as follows. The lid margin was divided into three equal sections (inner, middle and outer). Each section was graded on a 0-3 scale, where zero represents the line entirely on the conjunctival side of the meibomian orifices; 1 where any part of the line touches the orifices; 2 where the line runs through all orifices; and 3 where the line is located on the eyelid-margin side of the orifices. The overall score (maximum 9) was the sum of the three displacement scores for each lid margin section (Yamaguchi et al 2006) (Appendix J).

3.4.2.3.7 Tarsal Conjunctiva

The lower tarsal conjunctiva of the right eye was examined under 16x magnification (Bron et al 1991)for concretions and chalazia and scored 0 or 1 where zero represents normal (Appendix J).

3.4.2.3.8 Flow diagram of Clinical Tests Performed in the Study



3.4.2.4 Circulating Plasma Hormone Concentration

3.4.2.4.1 Venous Blood Collection, Processing and Storage

Venous blood collection was performed within one to 76 hours from the visit by a phlebotomist at UNSW Health Service. Nine ml of venous blood were drawn into a 9ml Ethylenediaminetetraacetic (EDTA) anticoagulant blood tube (Vacuette^{R}tube Greiner Bio-one, Austria). Blood sample tubes were transported on ice from the Health Service to the laboratory.

The plasma harvesting method was based on (Tuck et al 2008)

i. The blood was centrifuged for 15 minutes at 800 - 1200 g at 4°C
ii. The plasma layer above the buffy coat was collected and 1 ml aliquots were transferred using a sterile pipette into 5 Eppendorf tubes
iii. The plasma was stored at –80°C for 1 to 12 months prior to analysis
iv. Samples were thawed and inverted several times prior to testing

Circulating plasma concentration of total testosterone, oestradiol, progesterone, SHBG and DHEA-S were measured using enzyme-linked immunosorbent assay (ELISA) (DRG International, USA-NJ (2012-13 Manufacturer Protocol) and 3α-diolG was measured using a radioimmunoassay (RIA) (DIAsourceImmunoAssays S.A, Louvain-la-Neuve-Belgium) (2002 Manufacturer Protocol). Free testosterone was calculated with an online calculator requiring the input of total testosterone and SHBG (Vermeulen et al 1999). This was accessed at (http://www.issam.ch/freetesto.htm) from June 2012 to April 2014.

3.4.2.4.1.1 Procedure for ELISA

The ELISA kits for the studies in this thesis were chosen based on their sensitivity to the expected levels of the hormones and their availability in Australia. ELISA uses the principle of competitive binding where an enzyme detects the binding of antigen and antibody(Ma et al 2006).

Reagents including dilution buffer, wash solution, quality controls and master standards were prepared according to the manufacturer's protocol [DRG International, USA-NJ (2012-13)]. Undiluted plasma samples were used and the standards, quality controls and samples were pipetted into the appropriate wells of the ELISA plate in duplicate. The plate was incubated for the relevant time period and speed specified using the OM7orbitalmixer (Ratek, Australia), at room temperature and then washed three times with wash solution. The incubation period and speed was specific to each kit. After the excess wash solution was completely removed and the conjugate solution was added, the plate was incubated at room temperature on the OM7. The plate was washed as before three times and excess wash solution removed completely. The substrate solution was added and the plate incubated in the dark for 10 minutes or until a blue colour developed. The reaction was stopped by addition of stop solution into each well and the plate was read using a FLUOstar Omega microplate reader, BMG LABTECH

(Offenburg, Germany) at 450 nm within 5 minutes. The concentration of the samples was determined from the standard curve.

Table 3.1 Sex Hormones and Their Limits of Detection

Sex Hormones	Limits of Detection [DRG International, USA-NJ (2012-13)]
Oestradiol(EIA 2693)	9.7pg/mL
Total testosterone (EIA 1559)	0.08 ng/mL
SHBG (EIA 2996)	0.8nmol/L
Progesterone (EIA 1561)	0.05 ng/mL

3.4.2.4.1.2 Procedure for Radioimmunoassay (RIA)

Radioimmunoassay is a technique whereby antigen-antibody complexes were formed as a result of a competition between labelled and unlabeled antigen for distinct antibody sites(Goldsmith 1975). Two coated tubes in duplicate for each calibrator, sample and control were labelled. For the determination of total counts, two normal tubes were labelled. Calibrator, sample and control were briefly vortexed and 100 µl of each was dispensed into respective tubes. 0.5 ml of ^{125}Iodine labelled 3α-diolG was dispensed into each tube, including the uncoated tubes for total counts. The plate was incubated for 2 hours on the OM7orbitalmixer at 700 rpm at room temperature and then washed three times with wash solution. Excess wash solution was completely removed. After the last wash, the tubes were left to stand upright for two minutes. Tubes were counted in a gamma counter for 60 seconds. Table 3.2 displays the 3α-diolG measured and its limit of detection.

Table 3.2 Sex Hormone and Its limit of Detection

Sex Hormone	Limit of Detection DIAsourceImmunoAssays S.A,Louvain-la-Neuve - Belgium (2002)
3α-diolG(KIP0151)	0.2 ng/ml

3.4.3 **Statistical Analysis**

Data normality was assessed with using the Kolmogorov-Smirnov test (p>0.05). For the univariate analysis, associations between the study variables (ocular symptoms, sensitivity, plasma concentrations of sex hormones, ratios of sex hormones and clinical signs of dry eye) were simultaneously assessed with Pearson (parametric) and Spearman (non-parametric) Bivariate Correlation tests (p<0.05). Missing data were replaced with the group means of the respective variables. For multivariate analysis, determination of independent variables that predict ocular symptoms was performed using a general linear model (section 3.4.3.1 below). Mann-Whitney test and independent sample t-tests were used to examine the effects of gender on all variables (p<0.05). Standard Multiple Regression Analysis

Spearman and Pearson Bivariate Correlation tests were used as appropriate to examine associations between various ocular symptom scores and independent variables (sex hormone concentrations, ratios of sex hormones, tear volume, tear osmolarity, NIBUT, ocular surface sensitivity and staining, meibomian gland and lid margin assessments, age and contact lens wear). The ocular symptom score chosen as the dependent variable was based on the metric with the highest number of significant associations (p<0.25) from the univariate analysis. The independent variables associated with symptoms at p<0.25 were entered into a general linear model. The final model for significant variables was determined using the method of backward elimination followed by forward entry, and chosen based on optimising the R Square values, which gives an estimate of the percentage of the variance accounted for by the model. The independent variables in the final model were retained only if they were significant at p<0.05.

3.5 Results

Table 3.3 displays subjects' demographic variables. Normal to mild dry eye males, menstruating and postmenopausal women (54F:22M) within the age range of 19 - 76 years (mean 38 ± 14) were recruited.

Four subjects used hormone medication for thyroid disease, one female subject used homeopathic medication to regularize her menstrual cycle, nine females were using oral contraceptives containing either oestradiol such as Yasmin and Menofeme or the combination of oestrogen and progesterone such as Logynon and two postmenopausal women used hormone replacement therapy containing oestrogen such as Estradot.

Table 3.3 Subject Demographics

	All subjects	Male	Female	Contact Lens Wearers	Non-Contact Lens Wearers
n	76	22	54	37	39
Age (years)	35.0 ± 14.0	34.2 ± 13.8	36.3 ± 14.1	32.3 ± 10.3	38.9 ± 16.3
Ethnicity	33 Asian 43 Caucasian and others	10 Asian 11 Caucasian and others	24 Asian and 30 Caucasian and others	18 Asian 19 Caucasian and others	16 Asian 23 Caucasian and others

Others represent Africans and South Americans

3.5.1 **Normality of Age and Study Variables**

Table 3.4 displays the results of Kolmogorov-Smirnov test for normality of distribution. Variables with $p>0.05$ have a normal distribution. Normally distributed data are indicated in bold.

Table 3.4 Normality of Age and Study Variables in All Subjects (n=76), Males (n=22) and Females (n=54)

Variables	Kolmogorov-Smirnov (p values)		
	All Subjects	Males	Females
Age	p <0.001	0.03	p <0.001
Sex Hormone Levels			
Oestradiol (E2)	p <0.001	**0.10**	p < 0.001
Progesterone	p < 0.001	p < 0.001	p < 0.001
Total testosterone	p < 0.001	**0.18**	0.03
Free testosterone	p < 0.001	**0.20**	0.01
5alpha-androstane-3alpha and 17beta-diolglucuronide (3 α-diol G)	p < 0.001	p < 0.001	p < 0.001
Dehydroepiandrosterone Sulfate (DHEA-S)	**0.05**	**0.20**	**0.20**
Sex Hormone Binding Globulin (SHBG)	p < 0.001	**0.14**	p < 0.001
Oestradiol: total testosterone	p < 0.001	0.04	p < 0.001

Oestradiol: 5alpha-androstane-3alpha and 17beta-diolglucuronide (3 α-diol G)	p < 0.001	**0.08**	p < 0.001
Ocular Symptoms			
Ocular Surface Disease Index (OSDI)	p < 0.001	**0.13**	0.01
Ocular Comfort Index (OCI)	0.03	**0.20**	0.02
Subjective Evaluation Of Symptom Of Dryness (SESOD)	p < 0.001	0.01	p < 0.001
Dry Eye Questionnaire (DEQ) Frequency	0.03	**0.20**	**0.17**
Dry Eye Questionnaire (DEQ) Intensity	p < 0.001	**0.20**	0.01
Dry Eye Questionnaire (DEQ) Intensity AM	p < 0.001	**0.11**	p < 0.001
Dry Eye Questionnaire (DEQ) Intensity PM	p < 0.001	**0.20**	**0.06**
Dry Eye Questionnaire (DEQ) Bothersomeness	0.02	**0.20**	**0.20**
Dry Eye Questionnaire (DEQ) Total Score	**0.19**	**0.20**	**0.20**
Dry Eye Questionnaire (DEQ) 5	0.03	**0.04**	**0.12**
Numerical Ratings Questionnaire Comfort (NRSC)	p < 0.001	**0.11**	p < 0.001
Numerical Ratings Questionnaire Dryness (NRSD)	p < 0.001	**0.11**	p < 0.001
Numerical Ratings Questionnaire Foreign Body Sensation (NRSFB)	p < 0.001	p < 0.001	p < 0.001
Numerical Ratings Questionnaire Burning (NRSB)	p < 0.001	p < 0.001	p < 0.001
Numerical Ratings Questionnaire Watery (NRSW)	p < 0.001	0.01	p < 0.001
Ocular Surface Sensitivity			
Central Corneal Sensitivity (CCS) Right Eye	p < 0.001	p < 0.001	p < 0.001
Inferior Conjunctival Sensitivity (ICJS) Right Eye	p < 0.001	p < 0.001	p < 0.001
Clinical Signs			
Tear Osmolarity-Worst Eye	0.02	**0.20**	0.01
Non invasive Tear Break-Up Time (NIBUT)-Average for both eyes	p < 0.001	p < 0.005	p < 0.001
Tear Volume (Phenol Red Thread)-Average of both eyes	**0.09**	**0.20**	**0.16**
Corneal Staining-Average of both eyes	p < 0.001	p < 0.001	p < 0.001
Conjunctival Staining-Average of both eyes	p < 0.001	0.02	p < 0.001

Marx's Line Displacement -Lower Lid Right Eye	$p < 0.001$	$p < 0.001$	$p < 0.001$
Meibomian gland expressibility (secretion quality and gland expression) Lower Lid Right Eye	$p < 0.001$	$p < 0.001$	$p < 0.001$
Number of glands- Lower Lid Right Eye	$p < 0.001$	$p < 0.001$	0.02
Number of patent glands- Lower Lid Right Eye	$p < 0.001$	**0.20**	0.03
Number of Capped glands- Lower Lid Right Eye	$p < 0.001$	$p < 0.001$	$p < 0.001$

3.5.2 **Plasma Sex Hormone Concentrations**

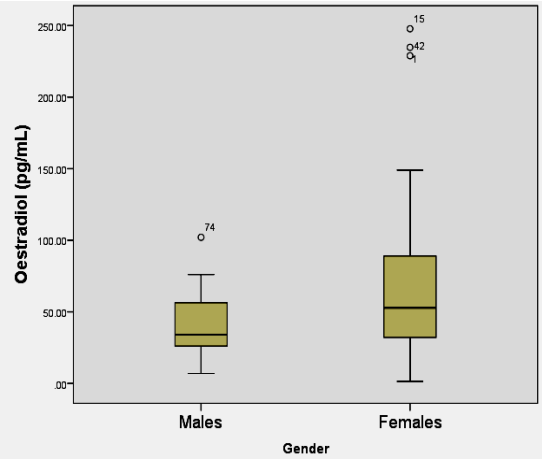
The ranges, inter quartile range and median for plasma concentrations of oestradiol, progesterone, total testosterone, free testosterone, DHEA-S, SHBG,3α-diolG, the ratio of oestradiol to total testosterone concentration and the ratio of oestradiol to 3α-diol G concentration in males and females are displayed in Table 3.5. Androgens, except for 3α-diol G, and progesterone concentrations were within normal published ranges. The maximum concentrations of oestradiol and SHBG exceeded the normal range for both genders (Figures 3.2 and 3.3) and the maximum concentration of 3α-diol G exceeded the normal range for females (Figure 3.4). As expected, the concentrations of oestradiol and progesterone were generally higher in females, while the concentrations of total testosterone, free testosterone, DHEA-S and 3α-diol G were higher in males.

Table 3.5 Sex Hormone Plasma Concentrations for Males and Females

Sex Hormones	Males n=22	Normative values (Males)	Females n=54	Normative values (Females)
	Ranges and (Inter Quarter Range/Median)			
Oestradiol: pg/mL	6.9 - 102.1 (33.9/29.4)	10 - 36*	1.4 - 247.8 (59/52.9)	11 - 191*
Progesterone: ng/mL	0.2 - 0.6 (0.2/0.3)	0.1 - 1.0*	0.2 - 26.3 (4.3/0.5)	0.1 - 25*
Total Testosterone: ng/mL	1.9 - 7.5 (2.7/3.5)	2.0 - 6.9*	0.2 - 1.2 (0.2/0.4)	0.3 - 1.2*
Free Testosterone: ng/mL	0.04 - 0.19 (0.1/0.1)	0.04 - 0.13 ŧ	0.001 - 0.01 (0.004/0.01)	0.001 - 0.006^
5alpha-androstane-3alpha and 17beta-diolglucuronide (3α-diolG) ng/mL	2.1 - 30.9 (3.4/6.2)	1.0 - 23.6*	0.1 - 14.3 (1.7/1.0)	0.1 - 7.9**
Dehydroepiandrosterone Sulfate (DHEA-S) µg/mL	0.7- 5.7 (1.9/1.8)	0.6 - 3.0*	0.3 - 4.2 (1.2/1.4)	0.4 - 2.2
sex hormone binding globulin (SHBG) nmol/L	11.5 - 94.8 (28.8/35.6)	10 - 57$^{\beta}$	19 - 284 (70.4/62.5)	18 - 144$^{\beta}$
Oestradiol: total testosterone	22 - 37.6 (8.7/7.7)	Not available	4.6 – 559 (116.9/109.4)	Not available
Oestradiol: 5alpha-androstane-3alpha and 17beta-diolglucuronide (3α-diolG)	11.7 - 27.6 (6.5/7.4)	Not available	0.9 – 643.6 (39.7/55)	Not available

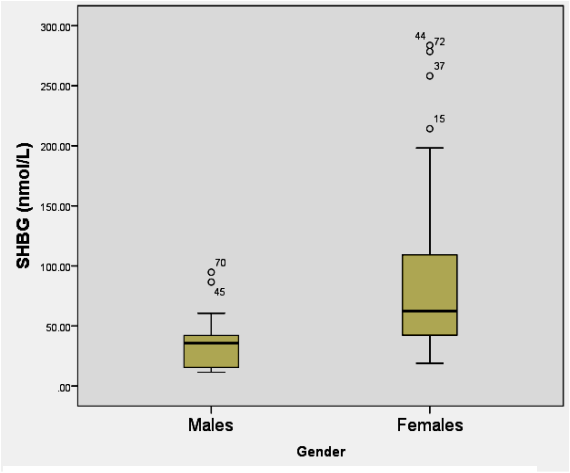
*(DRG International, USA-NJ (2012-13) Manufacturer Protocol
**DIAsourceImmunoAssays S.A, Louvain-la-Neuve - Belgium (2002) Manufacturer Protocol
^ (Braunstein et al 2011)
ŧ (Ho et al 2006)
β (Elmlinger et al 2002)

Figure 3.2 Oestradiol plasma concentration in males (n=22) and females (n = 54).



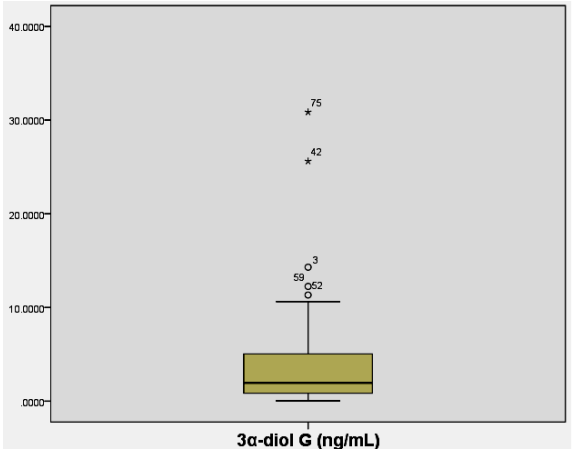
Circles represent the outliers of the plasma concentration of oestradiol belonging to the respective subjects with the identification number.

Figure3.3 Maximum concentration of SHBG exceeded the normal range for both gender and it was lower in males (n = 22) than females (n = 54).



Circles represent the outliers of the plasma concentration of SHBG belonging to the respective subjects with the identification number.

Figure 3.4 Maximum concentration of 3α-diol G exceeded the normal range for females (n = 54).



Circles represent the outliers of the plasma concentration of 3α-diol G belonging to the respective subjects with the identification number.

Table 3.6 displays the associations between plasma sex hormone concentrations and age in males and females. Oestradiol, total testosterone and DHEA-S were significantly reduced in females while free testosterone and progesterone were significantly reduced in males with age. There were no significant changes in other

hormone concentration and ratio of oestradiol to androgens with age. Significant associations are indicated in bold.

Table 3.6 Associations between Plasma Sex Hormone Concentrations and Age for Males and Females

Sex Hormone Concentration	Males (n =22)	Females (54)
Oestradiol: pg/mL	rho=-0.32, p= 0.16	rho**=-0.32, p= 0.02**
Progesterone: ng/mL	rho**=-0.53, p= 0.01**	rho=- 0.02, p= 0.91
Total Testosterone: ng/mL	rho=-0.18, p= 0.45	rho**=- 0.31,p=0.03**
Free Testosterone: ng/mL	rho**=- 0.44, p =0.04**	rho=-0.10, p= 0.46
5alpha-androstane-3alpha and 17beta-diolglucuronide (3α-diolG) ng/mL	rho=-0.29, p= 0.19	rho=-0.04, p= 0.77
Dehydroepiandrosterone Sulfate (DHEA-S): µg/mL	rho=-0.41, p= 0.06	rho**=- 0.37,p=0.01**
sex hormone binding globulin (SHBG): nmol/L	rho=- 0.36, p= 0.11	rho=-0.16, p= 0.26
Oestradiol: total testosterone	rho=- 0.19, p= 0.41	rho=- 0.07, p= 0.61
Oestradiol:5alpha-androstane-3alpha and 17beta-diolglucuronide (3α-diolG)	rho=- 0.01, p= 0.97	rho=- 0.21, p= 0.16

Significant inverse associations were shown between age and oestradiol; total testosterone; and DHEAS in females, while significant inverse associations were shown between age and progesterone; and free testosterone in males.

Significant negative associations between sex hormone concentrations with age in females and males (Figures 3.5 to 3.9) and the associations remained following removal of the outliers.

Figure 3.5 Association between total testosterone and age for females (n=54)

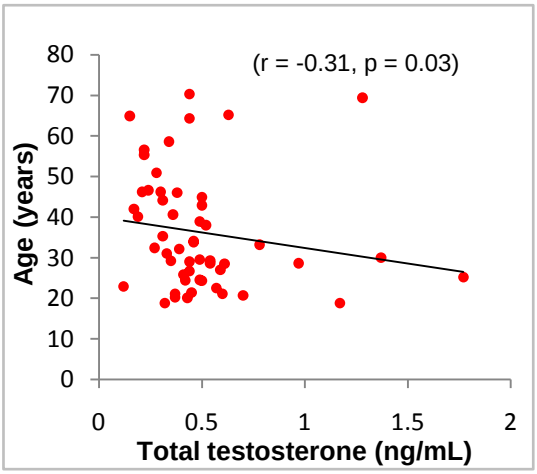


Figure 3.6 Association between oestradiol and age for females (n=54)

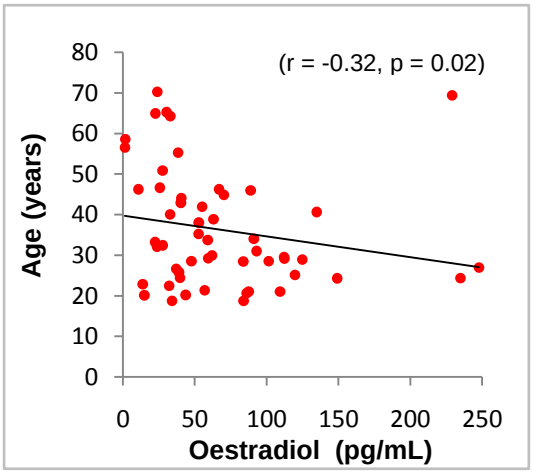


Figure 3.7 Association between DHEA-S and age for females (n=54)

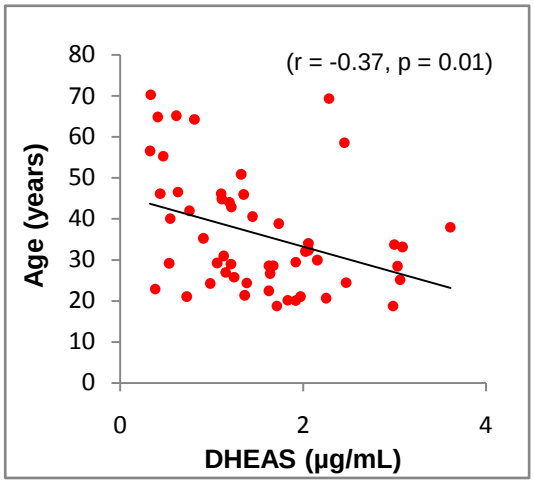


Figure 3.8 Association between progesterone and age for males (n=22).

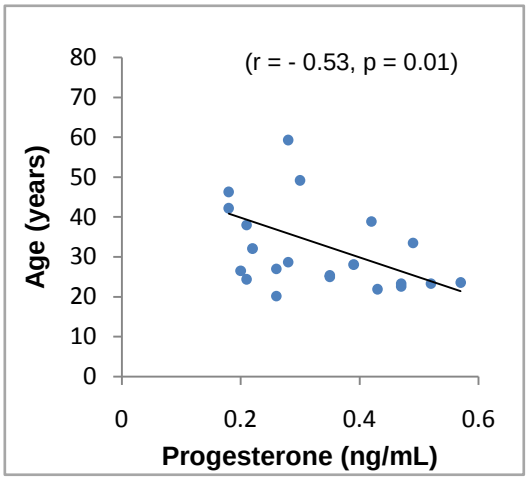
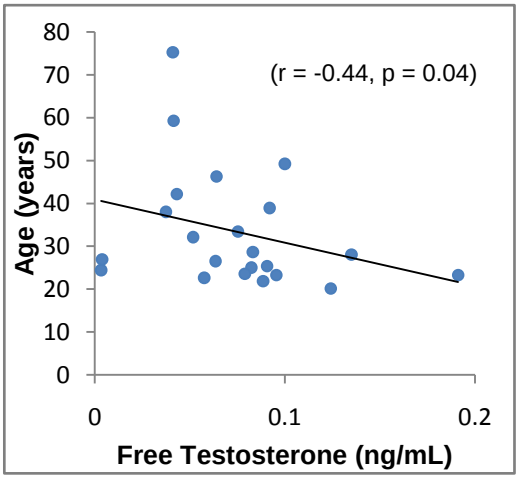


Figure 3.9 Association between free testosterone and age for males) (n=22)



3.5.3 **Ocular symptoms**

Mean group scores for all ocular symptom questionnaires were within a normal-to-mild dry eye classification (Table 3.7) since they were lower than the moderate dry eye scores in the OSDI, SESOD and DEQ 5. While the mean group symptom scores were higher for females for all questionnaires, there were no statistically significant differences in scores between genders. All questionnaires have a higher range of results in females. There were no significant associations between ocular symptoms and age across all groups (Appendices N, O and P).

Table 3.7 Ocular Symptoms Scores and Normative Values for All Subjects, Males and Females

Ocular Symptoms Questionnaires	Highest total score achievable	Male (n = 22)	Female (n = 54)	All Subjects (n = 76)	Normative values	p values between genders
		(Mean ± SD)				
Ocular Surface Disease Index (OSDI)	100	10.3 ± 9.7	15.2 ± 12.8	13.8 ± 12.1	Non dry eye (0 - 12) Mild dry eye (13 - 22) Moderate dry eye (23-32) (Miller et al 2010)	0.08
Ocular Comfort Index (OCI)	100	27.7 ± 6.4	29.6 ± 9.4	28.8 ± 8.8	Non dry eye (28 ± 5) (Jalbert et al 2012) Dry eye (> 40) (Evans et al 2009)	0.60
Subjective evaluation of symptom of dryness (SESOD)	4	0.9 ± 0.9	1.4 ± 1.0	1.3 ± 1.0	Asymptomatic Non dry eye (0 - 1) Symptomatic dry eye (2 - 4) (Srivinasan et al 2007)	0.28
Total Dry Eye Questionnaire Frequency (%)	100	18.5 ± 13.2	23.7 ± 15.3	22.2 ± 14.7	Not available	0.21
Dry Eye Questionnaire Intensity (A.M %)	100	13 ± 7.8	14.6 ± 14.9	14.2 ± 13.3	Not available	0.52

Dry Eye Questionnaire Intensity (P.M %)	100	21 ± 14.2	28 ± 21	.26 ± 19.5	Not available	0.11
Total Dry Eye Questionnaire Intensity (%)	100	17.0 ± 9.9	20.7 ± 15.8	19.5 ± 14.2	Not available	0.47
Total Dry Eye Questionnaire Bother (%)	100	20.3 ± 12.5	27.4 ± 19.8	25.2 ± 18.4	Not available	0.23
Total Dry Eye Questionnaire (%)	100	18.9 ± 11.1	25.2 ± 17.0	22.7 ± 15.4	Not available	0.17
Dry Eye Questionnaire (DEQ 5)	22	4.2 ± 4.3	6.8 ± 4.0	6.1 ± 4.2	2.7 ± 3.2 Non dry eye 8.6 ± 3.1 Mild dry eye 11.4 ± 3.3 Moderate 14.9 ± 2.3 Severe (Chalmers et al 2010)	0.41
Numerical Ratings Questionnaire Comfort	100	87.9 ± 9.1	87.9 ± 13.5	86.9 ± 12.3	Not available	0.48
Numerical Ratings Questionnaire Dryness	100	84.4 ± 17.5	78.2 ± 22.2	78.9 ± 21.7	Not available	0.52
Numerical Ratings Questionnaire Foreign Body Sensation	100	86.9 ± 20.3	82.4 ± 25.9	81.7 ± 25.7	Not available	0.24
Numerical Ratings Questionnaire Watery	100	91.6 ± 7.9	83.1 ± 24.5	84.1 ± 22.4	Not available	0.96
Numerical Ratings Questionnaire Burning	100	89.4 ± 24.6	86.7 ± 25.2	86.2 ± 26.3	Not available	0.31

NB: A high score with NRS indicates lower symptoms while high scores with other questionnaires indicate higher symptom

3.5.4 **Ocular Surface Sensitivity**

Group means for the central corneal and inferior conjunctival thresholds were within normal values (Table 3.8). There were no significant differences between genders. There were no significant associations between ocular surface sensitivity and age for all subjects, male and female (Appendices M, N and O).The means and standard deviations of ambient humidity and temperature recorded during the measurements were 53.7 ± 2.8% and 23 ± 2 ºC respectively.

Table 3.8 Ocular Surface Sensitivity and Normative Values for All Subjects, Males and Females

Ocular Surface	Males (n=22)	Females (n=54)	All subjects (n=76)	Normative Non dry eye	p values between genders
	(Means ± SD)				
Corneal sensitivity [1/g/mm^2]	2.4 ±0.4	2.0± 0.6	2.1± 0.6	2.7 ± 0.5 (Jalbert et al 2012)	0.12
Inferior Conjunctival sensitivity [1/g/mm^2]	0.7± 0.9	0.6± 0.8	0.6± 0.9	1.2 ± 1.1 (Jalbert et al 2012)	0.71

3.5.5 **Dry Eye Clinical Signs**

The scores for tear function, Marx's line displacement and meibomian gland expressibility assessments were within normal ranges (Table 3.9), while the ranges for ocular surface staining and the numbers of glands, capped and patent (expressible) glands were consistent with mild dry eye. The results were recorded as either mean and standard deviation or range for comparison with published normal values. Of the meibomian gland assessments, only the number of glands, the number of capped glands and the number of patent glands; meibomian gland expressibility (secretion quality and gland expression) and Marx's line displacement were analysed since these were the only variables with scores above 0 in these subjects.

NIBUT was slightly but not significantly higher in males while tear osmolarity, ocular surface staining and Marx's line scores were slightly but not significantly higher in females. Tear volume was significantly higher in males (p=0.04) (Figure 3.12).There were no other significant differences between genders. Tear break-up times in the first 25 subjects were recorded as the time to blink and not the time to tear film break up.

Thus, the remaining 51 participants were counselled to avoid blinking during the measurement and were given breaks. The results for the first 25 subjects were replaced with the mean of NIBUT of the subsequent 51 subjects for analysis.

Age was negatively associated with a reduced number of patent (secreting) glands (r= -0.25, *p*= 0.03) and increased tear osmolarity (r= 0.24, *p*=0.04) in all subjects. However there were no significant associations between age and other clinical signs. There were no significant associations between age and clinical signs in either males or females (Table 3.9). Bold figures represent the variable with significant difference between genders.

Table 3.9 The Clinical Signs and their Normative Values for All Subjects, Males and Females.

Variable	Males (n=22)	Females (n=54)	All subjects (n=76)	Non dry eye	p values between gender
	(Means ± SD/ Range)				
Tear Osmolarity (mOsms/L)	291.5 ±10.8	295.8 ±12.6	294.5 ±12.2	296.9 ±13.6 (Lemp et al 2011)	0.36
Tear Volume (PRT) (mm)	**20.7 ± 5.9**	**17.1 ± 5.8**	18.2 ± 6.1	19.7± 5.9 (Doughty et al. 2007)	**0.03**
Non-invasive break-up time NIBUT (sec)	11.9 ± 7.0	9.5 ± 4.5	10.2 ± 5.5	10 (Mengher et al 1985)	0.09
Corneal Staining (Grade)	0-3	0-4	0-4	0-2 (Bron et al 2003)	0.44
Inferior Conjunctival Staining (Grade)	0-2	0-4	0-4	0-2 (Bron, Evans et al. 2003)	0.87
Marx Line displacement	0.4± 0.8	1.4 ± 2.1	1.1 ±1.9	2.8 ± 1.6 (Yamaguchi et al 2006)	0.11
Meibomian Gland Expressibility	0-1	0-1	0-1	0-2 (Tomlinson et al 2011)	0.85
Number of glands/ capped glands/ patent glands	2-7/0-5/ 0-7	1-11/0-6/ 0-7	1-11/0-6/ 0-7	6-10/0/3-8 (Bron et al 1991)	0.73/0.51/ 0.52

The significant difference between genders is bolded.

Significant associations between the clinical signs and age for all subjects are graphed out in Figures 3.10 and 3.11.

Figure 3.10 Association between number of patent glands and age for all subjects (n=76)

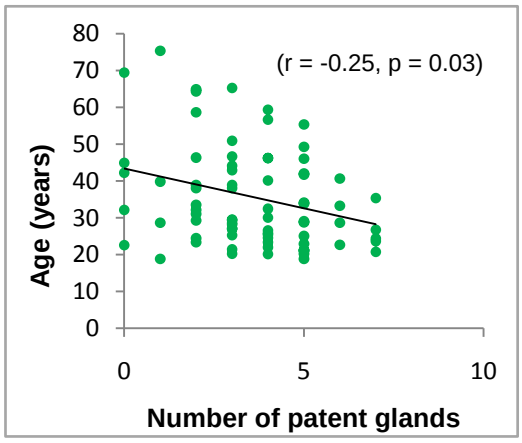


Figure 3.11 Association between tear osmolarity and age for all subjects (n=76)

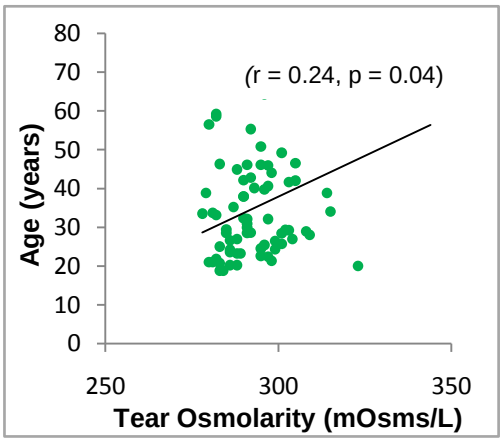
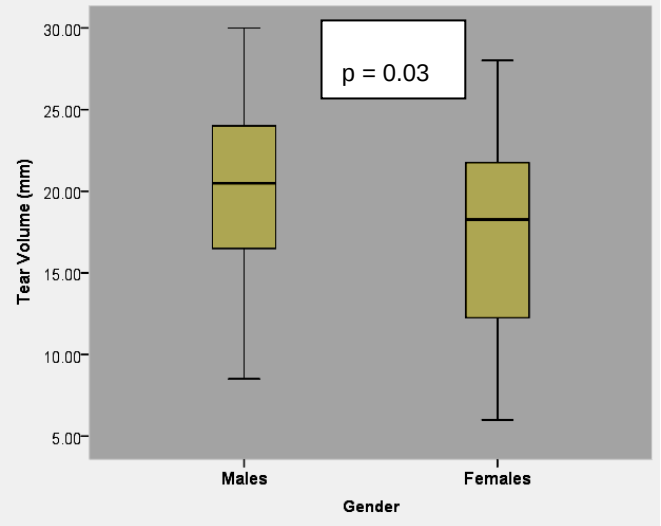


Figure 3.12 Tear volume (Phenol Red Thread) was significantly higher in males (n =22) than females (n = 54)



3.5.6 **Univariate Analysis**

Associations in between plasma sex hormone levels, ratios of oestradiol to androgens and ocular symptoms, ocular surface sensitivity, clinical signs, age and contact lens wear in 'all subjects', 'all females' and 'all males' are presented in Appendices K to S.

3.5.6.1 Associations between Plasma Sex Hormone Concentrations and Dry Eye Symptoms and Signs

3.5.6.1.1 Associations between Plasma Sex Hormone Concentration and Symptoms

A higher level of free testosterone and total testosterone was positively associated with lower scores for OSDI, OCI and DEQ questionnaires in all subjects and in 'males only' (Figures 3.13 to 3.15 and 3.18 and 3.19). A higher level of oestradiol was positively associated with higher scores of NRS foreign body and dryness and DEQ 5 in all subjects and in females (Figures 3.16 to 3.17).The ratio of oestradiol to total testosterone was positively associated with higher scores of OSDI in all subjects (Figure 3.20). There were no significant associations between sex hormone concentrations, ratio of oestradiol to 3α-diol G; and other ocular symptoms scores. The result remained consistent with the removal of the outliers (Figures 3.16 to 3.20).

Figure 3.13 Association between total testosterone and Ocular Surface Disease Index score in all subjects (n=76)

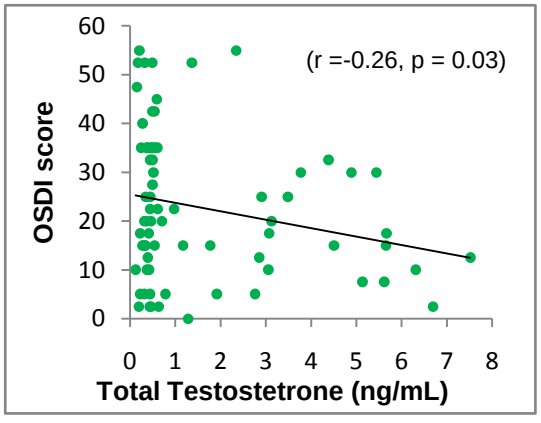


Figure 3.14 Association between free testosterone and Ocular Surface Disease Index score in all subjects (n=76)

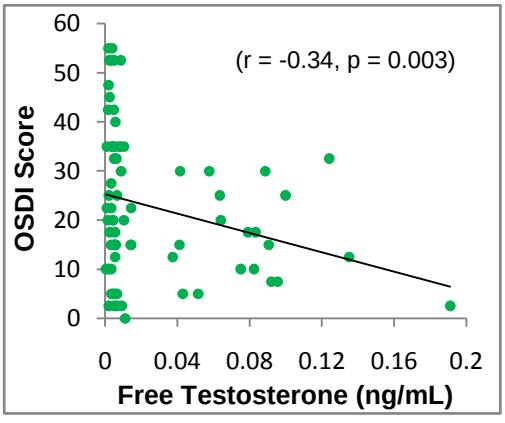


Figure 3.15 Association between free testosterone and Ocular Comfort Index score in all subjects) (n=76)

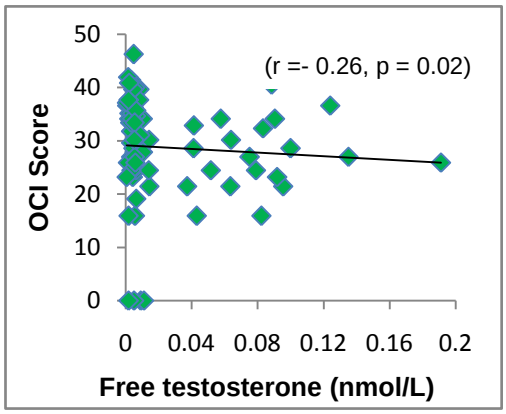


Figure 3.16 Association between oestradiol and Dry Eye Questionnaire 5 score in all subjects (n=76)

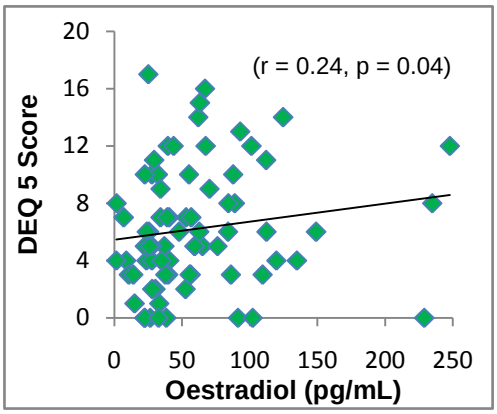
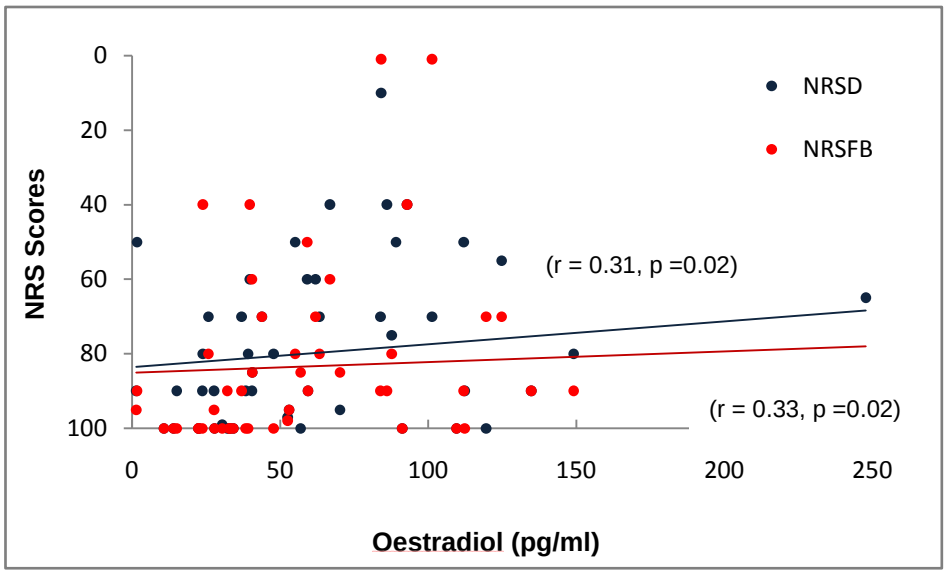


Figure 3.17.Association between oestradiol and Numerical Rating Questionnaires Dryness (NRSD) and Numerical Rating Questionnaires Foreign Body Sensation (NRSFB) in females (n=54)



NB: A high score with NRS indicates lower symptom

Figure 3.18 Association between free testosterone and score of Dry Eye Questionnaire (DEQ) Frequency, DEQ "Bothersomeness" and DEQ Total in all subjects (n=76).

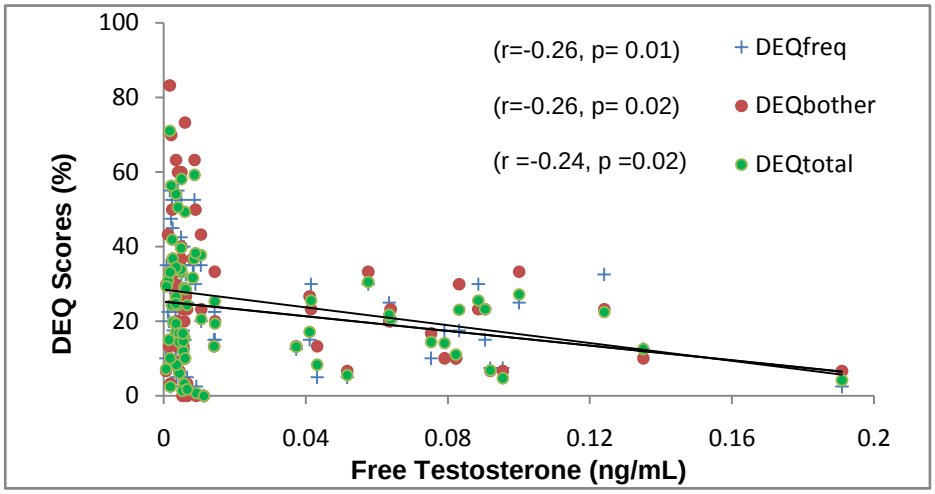


Figure 3.19 Association between free testosterone and Dry Eye Questionnaire (DEQ) Total and DEQ "Bothersomeness" of symptoms in males (n=22).

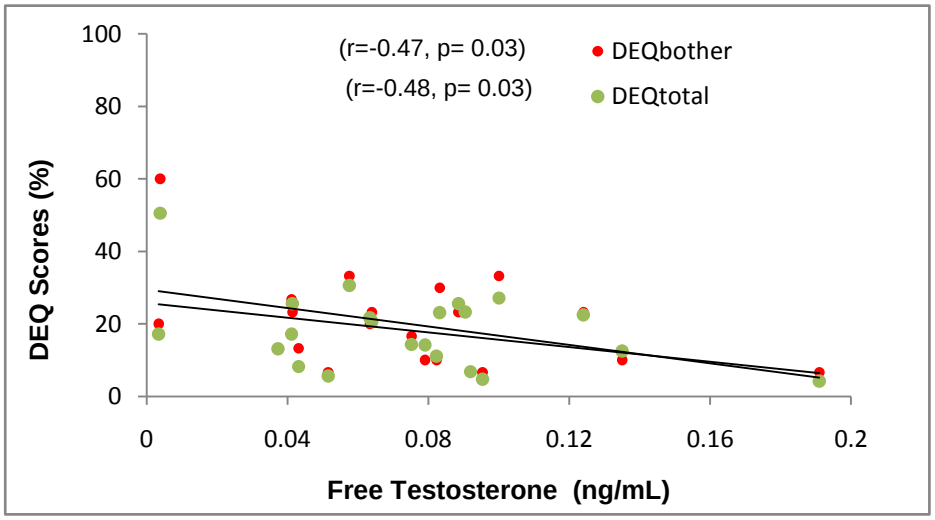
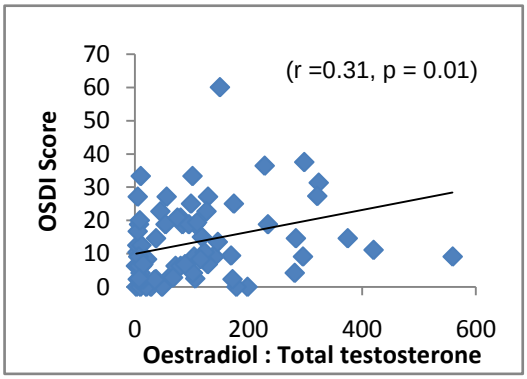


Figure 3.20 Association between the ratio of oestradiol to total testosterone and Ocular Surface Disease Index in all subjects (n=76).



3.5.6.1.2 Associations between Sex Hormone Concentrations and Ocular Surface Sensitivity

A higher level of 3α-diol G and free testosterone was significantly associated with higher corneal sensitivity in all subjects and in males (Figures 3.21 and 3.22). There were no significant associations between other sex hormone concentrations, ratios of oestradiol to androgens and either corneal or conjunctival sensitivity. There were no significant associations between sex hormone concentrations and ocular surface sensitivity in females. The result remained consistent with the removal of the outliers.

Figure 3.21 Association between free testosterone and corneal sensitivity in males (n=22)

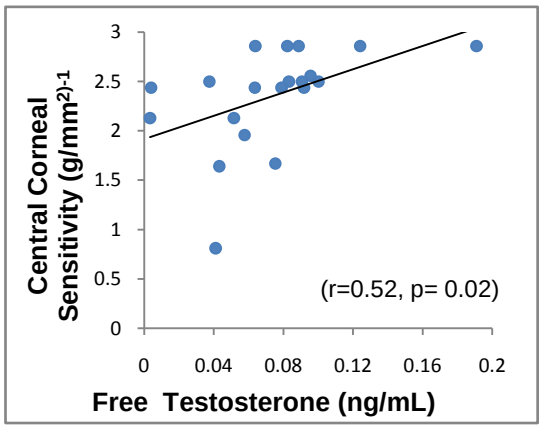
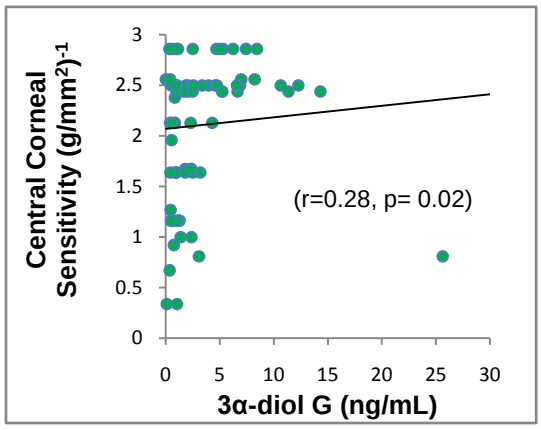


Figure 3.22 Association between 3α-diol G and corneal sensitivity in all subjects (n=76)



3.5.6.1.3 Associations between Sex Hormone Concentrations and Dry Eye Clinical Signs

A higher level of 3α-diol G was negatively associated with higher tear volume in all subjects and in females, and with a higher number of patent glands in males (Figures 3.23 to 3.25). A higher ratio of oestradiol to total testosterone was negatively associated with tear volume in all subjects (Figure 3.26). A higher level of DHEA-S was associated with a lower tear osmolarity in all subjects and in females; and with a lower score of Marx's line position in males (Figures 3.27 to 3.29). However in females, a higher level of free testosterone was associated with a greater displacement of Marx's line (Figure 3.30).The ratio of oestradiol to total testosterone was positively associated with tear volume in all subjects (Figure 3.31). There were no significant associations between other sex hormone concentrations, ratio of oestradiol to 3α-diol G and other clinical signs.

Figure 3.23 Association between the 3α-diol G and tear volume in females (n=54)

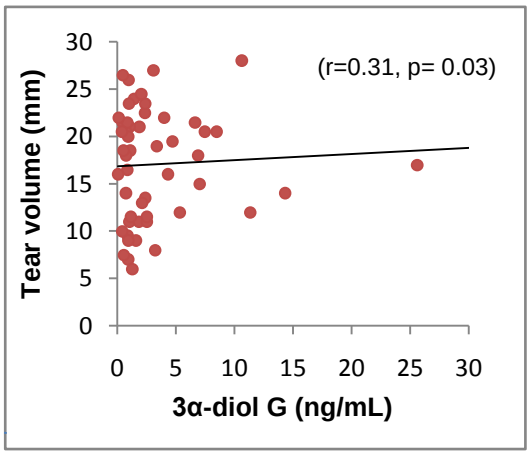


Figure 3.24 Association between the 3α-diol G and number of patent glands in males (n=22)

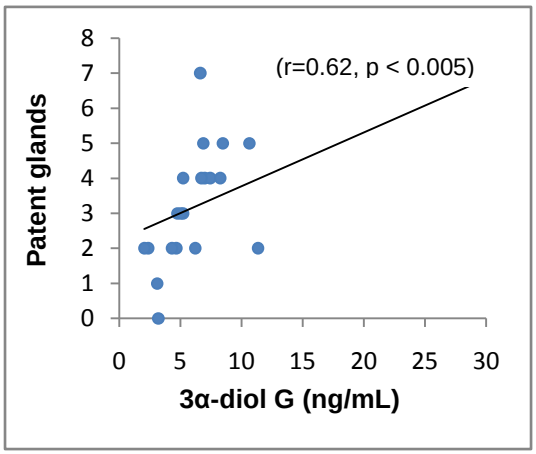


Figure 3.25 Association between the 3α-diol G and tear volume in all subjects (n=76)

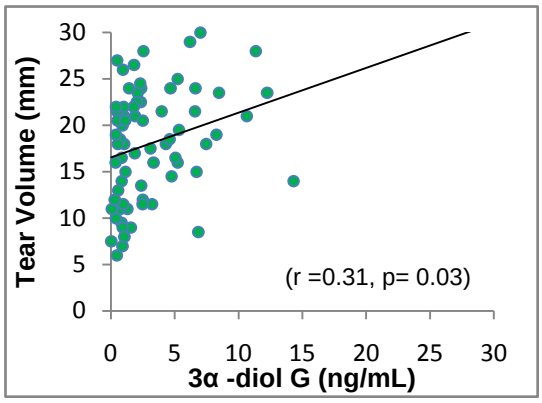
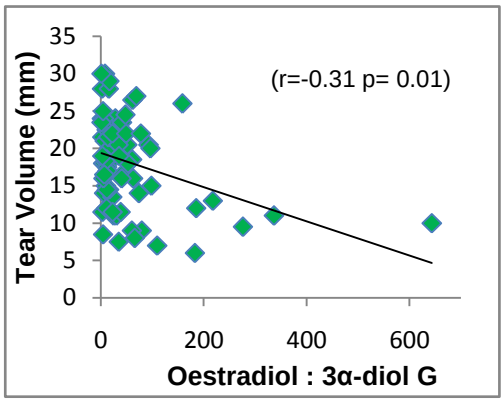


Figure 3.26 Association between the ratio of oestradiol to 3α-diol G and tear volume in all subjects (n=76)



3.27 Association between the ratio of oestradiol to total testosterone and tear osmolarity in all subjects (n=76)

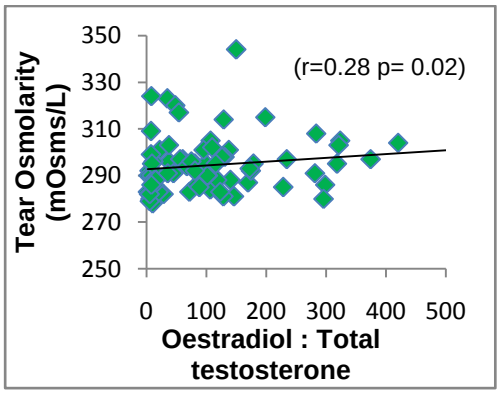


Figure3.28 Association between DHEAS and tear osmolarity in all subjects (n=76)

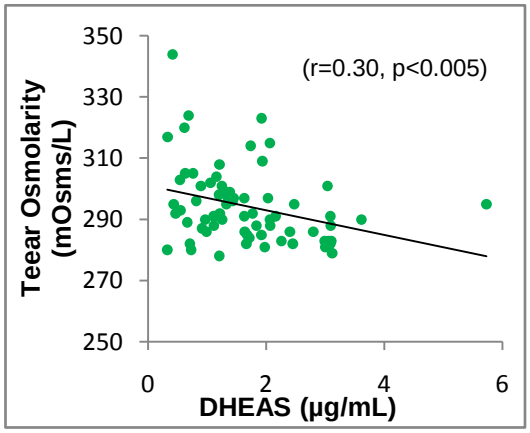


Figure 3.29 Association between DHEAS and Marx line in males (n=22)

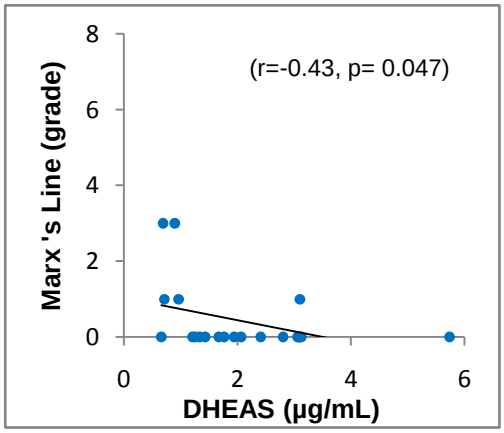


Figure 3.30 Associations between DHEAS and tear osmolarity in females (n=54)

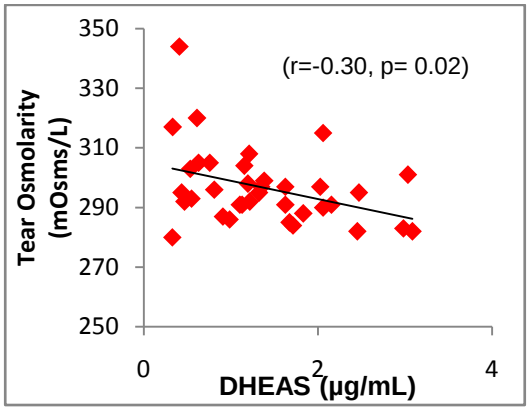
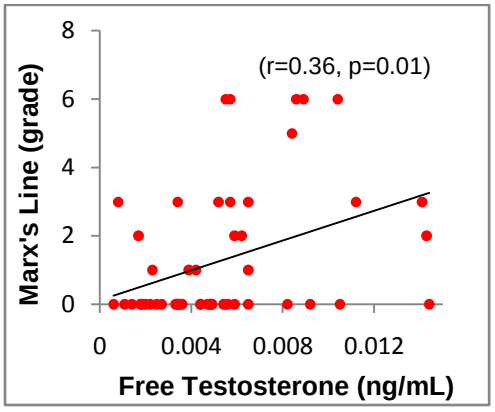


Figure 3.31 Association between the free testosterone and Marx line in females (n=54)



3.5.7 Multivariate Analysis to Identify Predictors of Ocular Symptoms

3.5.7.1 All Subjects

DEQ 5 was chosen as the outcome measure because it was deemed to be representative of the dry eye status of the participants. Significant univariate relationships were observed between DEQ 5 score and age; contact lens wear; oestradiol; free testosterone; ratio of oestradiol to 3α-diol G; ratio of oestradiol to total testosterone; inferior conjunctival sensitivity; corneal and conjunctival staining, NIBUT and tear volume. These relationships were analysed to form a model. Variables were selected to form the model based on their significant associations with the DEQ 5 score (p<0.25) in the univariate analysis. The final model contained age, oestradiol; the ratio of oestradiol to total testosterone; inferior conjunctival sensitivity; NIBUT; conjunctival staining and tear volume (Table 3.10). The model explained 24.2% of the variance in DEQ 5 and was statistically significant (p=0.01). The equation generated for DEQ 5 is = - 0.19 NIBUT + 11.74 (bold in Table 3.10). With a lower NIBUT score, the DEQ 5 scale was expected to be higher (more symptoms) after controlling for the other variables in the model.

The constant appears at the top of the unstandardized coefficient table output of a model, when the selected independent variables are included in the regression analysis. Constant will **NOT** affect the association between the dependent (DEQ

5) and independent variables (NIBUT). However constant is important in making sure that the prediction of these associations between DEQ 5 score and the rest of the independent variables are unbiased. In this model, a decrease of one second of NIBUT increases the DEQ 5 score by 0.19 unit.

Table 3.10 The Multivariate Analysis on Ocular Surface Symptoms in All Subjects (n=74).

Dependent variable	**DEQ5**	
Significant univariate relationships at p < 0.25	Age	
	contact lens wear	
	Oestradiol	
	Free Testosterone	
	Oestradiol:3α-diol G	
	Oestradiol:Total testosterone	
	inferior conjunctival sensitivity	
	corneal staining	
	conjunctival staining	
	NIBUT	
	tear volume	
Independent variables in the final model	**Unstandardised Coefficients β**	**p value**
	11.74 Constant	0.00
	-0.06 Age	0.08
	-0.01 Oestradiol	0.46
	0.01 Oestradiol:Total testosterone	0.15
	-0.57 inferior conjunctival sensitivity	0.27
	0.46 conjunctival staining	0.34
	-0.19 NIBUT	**0.04**
	-0.1 tear volume	0.24
R^2%	24.2	
p value	0.01	
Predictor/s	-0.19 NIBUT	
Equation	DEQ 5 = -0.19 NIBUT + 11.74	
Unstandardized Coefficients column with significant p values (p<0.05) are bolded		

3.5.7.2 All Females

The multivariate analysis was also performed on DEQ 5 based on the similar justification as in all subjects. Significant univariate relationships were observed between DEQ 5 with age; oestradiol; ratios of oestradiol to androgens; corneal and inferior conjunctival sensitivity; corneal and conjunctival staining, NIBUT and tear

volume were analysed to form a model. The final model contained all of the initial independent variable. The model explained 36.2% of the variance in DEQ 5 and was statistically significant (p=0.02) (Table 3.12). The equation generated for DEQ 5 is = -1.31 inferior conjunctival sensitivity – 0.27 NIBUT + 7.45 (bold in Table 3.11). With lower inferior conjunctival sensitivity and NIBUT scores, the DEQ 5 scale was expected to be higher (more symptoms) after controlling for the other variables in the model.

Constant appears at the top of the unstandardized coefficient table output of a model, when the selected independent variables are included in the regression analysis. Constant will **NOT** affect the association between the dependent (DEQ 5) and independent variables (Inferior conjunctival sensitivity). However constant is important in making sure that the prediction of the associations between DEQ 5 score and the rest of the independent variables are unbiased. In this model, a decrease of one second of NIBUT increases the DEQ 5 score by 0.27 unit and a decrease of one ($1/(g/mm^2)$) of inferior conjunctival sensitivity increases the DEQ 5 score by 1.31 unit.

Table 3.11 The Multivariate Analyses on Ocular Surface Symptoms in Females (n=54).

Dependent variable	**DEQ5**	
Significant univariate relationships with	Age	
	Oestradiol	
	Oestradiol : 3α- diol G	
	Oestradiol: total testosterone	
	corneal sensitivity	
	conjunctival sensitivity	
	corneal staining	
	conjunctival staining	
	NIBUT	
	tear volume	
Independent variables in the final model	**UnstandardisedCoefficients β**	**Significant p value**
	7.45 Constant	**0.00**
	-0.02 Age	0.59
	-0.02 Oestradiol	0.35
	< 0.001 Oestradiol : 3α- diol G	0.97
	0.01 Oestradiol: total testosterone	0.15
	1.55 corneal sensitivity	0.08
	-1.31 Inferior conjunctival sensitivity	**0.048**
	0.38 corneal staining	0.53
	0.19 conjunctival staining	0.75
	-0.27 NIBUT	**0.03**
	-0.05 tear volume	0.63
R^2%	36.2	
p value	0.02	
Predictor/s	-1.31 inferior conjunctival sensitivity-0.27 NIBUT	
Equation	DEQ 5 = -1.31 inferior conjunctival sensitivity-0.27 NIBUT + 7.45	

Unstandardized Coefficients column with significant p values ($p<0.05$) are bolded

3.6 Discussion

3.6.1 General Findings

In this normal-to-mild dry eye population, several associations have been established which may help to understand the relationships between dry eye and sex hormones. As expected, the ranges of female sex hormones (oestradiol and progesterone) were generally higher in females while the ranges of male sex hormones (androgens) were higher in males. Sex hormone binding globulin (SHBG) level was within normal range in both genders. There were occasional outliers, but these did not materially change the reported associations. Furthermore, no disease or disorders that might have been related to the variations in hormone levels were recorded in the subject information data.

In the univariate analysis, In the univariate analysis, oestradiol was positively associated with dry eye symptoms. However, no significant association was recorded between oestradiol and other parameters. Androgens were negatively associated with symptom, tear osmolarity and Marx's line score but however were positively associated with ocular surface sensitivity; tear volume and number of patent gland.

In the multivariate analysis, although oestradiol and the ratios of oestradiol to androgens were in the final model, they did not have predictive capability of symptoms in the presence of other independent variables. Evidently, in this population, conjunctival sensitivity and staining; and NIBUT were the predictors of symptoms.

In the current study, the effect of gender on dry eye was only observed in tear volume and the effect of aging on dry eye was represented by the reduction in the sex hormones concentrations; number of patent (lipid secreting) glands and increased in tear osmolarity.

3.6.2 **Ocular Symptoms**

3.6.2.1 Univariate Analysis

Ocular symptoms might be affected by sex hormones. Free testosterone and 3α-diol G were negatively associated with symptoms in ‘all subjects’ and ‘males only’. These associations might be due to the contribution of higher concentration of testosterone in males in both groups. Oestradiol was positively associated with symptoms in ‘all subjects’ and ‘females only’ which were likely due to the higher concentration of oestradiol in females in both groups. In addition, higher symptoms were also associated with a higher ratio of oestradiol to total testosterone in ‘all subjects’ and ’females only’. These findings support the thesis hypotheses of dry eye symptoms associating positively with higher oestrogen and lower androgen concentration.

3.6.2.2 Multivariate Analysis

The importance of relationship between sex hormones and dry eye symptoms was further indicated with the presence of oestradiol, and the ratios of oestradiol to androgens as factors in the final models of the multivariate analysis in the ‘all subjects’ and the ’females only’ groups. The ratios of oestradiol to androgens showed a positive relationship with symptoms, supporting the potential for an increase in symptoms with higher concentration of oestradiol and/or lower concentration of circulating androgen as hypothesised. However, the positive association between oestradiol and symptoms in univariate analysis was “transposed” by the suppressor effect, which is influenced by the β weight coefficient (Conger 1974),to a negative association in the multivariate analysis.β weight coefficient defines the change in the dependent variable with a one-unit change in the predictor variable, holding all other predictor variables constant (Courville & Thompson 2001) since β weights are quite unstable (Conger 1974). As a suppressor variable, the oestradiol demonstrated a change in the direction of the coefficient after receiving the unexpected negative regression β weight in the multivariate analysis. However, neither oestradiol nor the ratio of oestradiol to androgens was significantly associated with symptoms and did not have predictive capability of symptoms in the presence of the other independent variables. Sex

hormones were also not established in the literature review as the significant independent variable of symptoms in females who were mostly symptomatic (Mathers et al 2002).

The current study demonstrated that ocular symptoms were predicted by the conjunctival sensitivity and staining, and NIBUT. Lower conjunctival staining and higher scores for both inferior conjunctival sensitivity and NIBUT were significantly associated with lower symptoms. It is interesting to note that NIBUT appeared as the significant predictor of symptoms in both groups of 'all subjects' and 'females only' which proved this clinical sign as the important indicator of dry eye symptoms. However, there were two confounding factors which might have affected the NIBUT measurements. Firstly, the first 25 NIBUT measurements were replaced with the means of the subsequent 51 measurements due to the differences in the ways the samples were measured. Therefore, the actual readings were not included. Secondly, the analysis was performed on a single set of data which included both contact lens wearers and non-wearers. Contact lens wear might impact tear film stability and hence NIBUT by affecting the biophysical and biochemical properties of tears (Craig et al 2013) in the current study. The inclusion of subjects' original measurement of NIBUT and separate NIBUT results between contact lens wearer and non-wearers should be considered to obtain a more reliable association with ocular symptoms in future studies.

Tear osmolarity was among the factors listed in the final model which however was not a consistent predictor of symptoms, although it has been shown as the best clinical sign to diagnose and classify dry eye disease (Lemp et al 2011). This is possibly because, although tear osmolarity is exceptionally good at differentiating normal subjects from severe dry eye (Lemp et al 2011), it is less robust in distinguishing normal subjects from mild dry eye. TBUT was more effective in identifying the early stage and mild dry eye subjects (Lemp et al 2011) who were among the subjects in the current study.

NIBUT and both corneal and conjunctival sensitivity were also significant predictors of symptoms (OSDI) (Situ et al 2008a) indicating the importance of ocular surface sensitivity as a dry eye clinical indicator. However, only the conjunctival sensitivity and not the corneal sensitivity that was significantly associated with symptoms in the

current study. This finding is generally consistent with a previous study showing a more significant change in the conjunctiva than the cornea in dry eye (Situ et al 2008b). This is consistent with the idea that the conjunctiva is directly involved in the regulation of aqueous and mucin phases of the tear film and covers a wide area of the ocular surface (Situ et al 2008b). In the current study, the conjunctival sensitivity was positively associated with corneal sensitivity. However, the corneal sensitivity was positively associated with symptoms, against the direction of association between the conjunctival sensitivity and symptoms. These inconsistent findings require further investigations to explore the relationships between ocular surface sensitivity and ocular symptoms.

Age was an important factor affecting the symptoms which appeared in the final models. However, age was negatively associated with symptoms in contrast to the association shown in the univariate analysis which might be due to the β weight effect as explained above.

Ocular surface staining and tear volume were among the factors in the final model which were not consistent predictors of symptoms There are numerous studies showing significant associations between ocular symptoms and signs (Adatia et al 2004, Afonso et al 1999, Cennamo et al 2007, Gulati et al 2006, Macri & Pflugfelder 2000, Ozcura et al 2007, Tuisku et al 2008) in contrast to one study which found a weak (Hay et al 1998) or no association between ocular symptoms and clinical signs (Nichols et al 2004, Sullivan et al 2014). In the current study, age, oestradiol concentration, the ratios of oestradiol to androgens, tear function and; ocular surface sensitivity and integrity contributed to the variance of the regression analysis in symptoms prediction.

3.6.2.3 Effect of Age and Gender on Ocular Symptoms

As anticipated, females showed higher symptom scores than males for all questionnaires. However; this effect was not statistically significant.

Age may or may not affect ocular symptoms. There was no linear relationship between age and symptoms in this study, consistent with a previous epidemiology study (Schein et al 1997b) with the latter study proposing the insensitivity of Schirmer and

rose bengal tests in identifying subjects with symptoms. However, both of the tests were not performed in the current study. In contrast, several epidemiological studies showed increasing symptoms with age (Lee et al 2002, Lin et al 2003, McCarty et al 1998, Moss et al 2008). All of these epidemiology studies, except one, were performed in an older population.

3.6.3 **Ocular Surface Sensitivity**

Ocular surface sensitivity might be affected by sex hormones. In the current study, higher level of free testosterone and 3α-diol G were associated with increased corneal sensitivity in males. A lower testosterone level might disrupt the normal regulation of the lacrimal gland and cause tissue damage, leading to a reduced tear flow, as in keratoconjunctivitis sicca (O'Brien & Collum 2004), decreased washout and removal of surface debris resulting in a longer residence time for inflammatory cytokines (Mathers 2000). It has been speculated that such inflammatory cytokines inhibit parasympathetic neural transmission in the peripheral nerves which eventually results in fewer signals being received by the lacrimal gland (Schäfer et al 1994)and hence reduces surface sensitivity in chronic dry eye disease. The association between central corneal sensitivity and free testosterone and 3α-diol G supports the hypothesis that a sufficient level of androgen (Labrie et al 2003)at the ocular sites is necessary to maintain normal homeostasis and a sufficiently lubricated and healthy ocular surface(Mathers 2000, Stern et al 2004, Sullivan et al 2000). Ocular surface sensitivity may be affected in dry eye by the androgen and oestrogen hormone-receptor activation on the ocular surface or indirectly through the neural feedback loop, linking the lacrimal gland and ocular surface (Mathers 2000, Stapleton et al 2013).

The precise sequence of events linking changes in ocular surface sensitivity, tear production and lacrimal gland stimulation is not fully understood. However it is likely that some sort of feedback loop connects the actions of all three components. Under normal circumstances therefore, ocular surface sensitivity regulates lacrimal gland stimulation leading to alterations in tear production (Mathers 2000, Stapleton et al 2013). The extent to which ocular surface sensitivity can be up or down regulated on the basis of changes to lacrimal gland stimulation and resulting tear volumes, remains to be determined. This observation might occur only in males in the current study due to

the higher concentration of androgen in males relative to females. Apart from this, the differences in the regulation of genes and in the number of hormone receptors are also present between genders. Among the 295 lacrimal genes which appeared to be regulated by androgens in male and female mice, 71 are induced (the majority in males) and 224 are suppressed (the majority in females) by androgen (Sullivan et al 1984). Furthermore, the number of androgen receptors on the lacrimal gland is greater in male rats compared to female rats (Rocha et al 1993, Sullivan et al 1996). Although many of the genes modulated by testosterone in female lacrimal and meibomian glands are identical to those regulated by androgens in male tissues, there are a few genes down regulated only in the female lacrimal gland (Sullivan et al 2009). The affected genes maintain immunity of the tissue (Kampa et al 2008)and are speculated to modulate the intracellular calcium release (Brown et al 1995) which regulates the life of cells (Marks 1997). Therefore, the down regulation of these functions by testosterone may contribute to dry eye in females, particularly during pre-menopause as previously demonstrated (Mathers et al 1998). Nevertheless, the inferior conjunctival sensitivity was among the significant predictors of symptoms, revealing the importance of ocular surface sensitivity as an important dry eye clinical indicator.

3.6.3.1 Effect of Age and Gender on Ocular Surface Sensitivity

Although ocular surface sensitivity measurements were within a normal range, there were no consistent associations between this clinical indicator and age while gender has no effect on the measurement, as opposed to previous investigations using the non-contact aesthesiometer (Acosta et al 2006, Bourcier et al 2005, Golebiowski et al 2008, Situ et al 2008b) and contact aesthesiometer (Millodot 1977a). Air jet and contact aesthesiometers differ markedly in composition of the stimulus and mode of stimulation and are likely to assess different aspects of the neural response (Golebiowski et al 2011) and hence resulted in the lack of consistent associations in the current study. The air-jet aesthesiometer Non Contact Corneal Aesthesiometer was also more reliable than the Cochet–Bonnet aesthesiometer (Murphy et al 1998). In addition, a larger sample size (such as n = 205 used in Millodot et al) might have allowed the significant association between age and corneal sensitivity to be observed compared to the current study (n = 76). The current study might not have been sufficiently powered to detect such significant association.

3.6.4 **Dry Eye Clinical Signs**

The positive effects of androgen on the lacrimal and meibomian glands and other components of the ocular surface have been demonstrated or suggested in numerous previous studies in both humans and animals (Cermak et al 2003, Khandelwal et al 2012, Krenzer et al 2000, Labrie et al 1997, Mamalis et al 1996, Mathers & Lane 1998, Sullivan et al 2002a, Sullivan et al 2002b, Sullivan 2004a, Sullivan 2004b, Sullivan et al 1990, Sullivan et al 2009, Sullivan et al 2002c, Sullivan et al 2000, Thody & Shuster 1989)**.** In the current study, higher androgen levels were associated with higher tear volume and number of patent glands but a lower tear osmolarity and lower Marx's line displacement. The identification of androgen and oestrogen receptor mRNAs (Wickham et al 2000) and their steroidogenic enzymes (Schirra et al 2006) in meibomian and lacrimal glands suggest the mechanism behind these associations. Androgens act on the acinar epithelial cells in these ocular tissues, which contain receptor mRNA and/or androgen receptor protein (Rocha et al 2000, Sullivan 2004b).

3.6.4.1 Meibomian Gland

In the meibomian gland, the acinar epithelial cells respond to androgens by transcribing specified genes to increase lipid production (Sullivan et al 2000). With sufficient lipid in the tear film layer, tear evaporation is reduced and tear osmolarity maintained. This was consistent with the negative associations between DHEA-S, as androgen precursor, and tear osmolarity in 'all subjects' and in 'females only' in the current study. The result was recorded in 'females only' which may be due to DHEA-S being the most abundant sex hormone in females, as supported by Panjari and Davis (Panjari & Davis 2007),and might have also driven the results in 'all subjects'.

The association between androgen and lower Marx's line displacement (Marx 1924) was demonstrated in the current study. In a healthy eye, this line runs along the inner eyelid and located on the conjunctival side of the meibomian orifices, but can be displaced towards the cutaneous side of the meibomian orifices in disease states (Yamaguchi et al 2006). The location of Marx's line gives an indication of meibomian gland function (Yamaguchi et al 2006) and displacement of Marx's line was negatively associated with DHEA-S in males but was positively associated with free testosterone in females. The androgen-related associations in the current study further suggest the

importance of androgen in maintaining meibomian gland function (Sullivan et al 2002c, Sullivan et al 2000). Mathers et al suggested that tear function worsened with testosterone in premenopausal women (Mathers & Lane 1998) which is consistent with the association between free testosterone and Marx's line displacement where the majority of the female subjects in the current study were premenopausal women. However, the study was not designed with sufficient power to test this particular hypothesis but this would be relevant to explore in future studies.

3.6.4.2 Lacrimal Gland

The acinar cells of the lacrimal gland bind androgen to a specific lipid producing area on the cell to initiate the lacrimal gland-androgen mechanism in tear production (Rocha et al 2000, Sullivan 2004b). Apart from this classical androgen-receptor pathway (direct combination between androgens and their receptors), androgens might also act by binding SHBG, which then binds its receptor (R SHBG). This in turn will activate cyclic adenosine monophosphate (cAMP) protein kinase A and regulate protein transcription in the lacrimal gland (Michels & Hoppe 2008). A similar mechanism appears to be operating in the present study in both males and females where it is conceivable that a higher level of 3α-diol G would be associated with a greater tear volume. The mechanism behind this hormonal activity is not fully understood but might be explained in part by the induction of significant and positive effects on the secretory process in the lacrimal gland (Sullivan 2004a).

3.6.4.3 Effect of Age and Gender on Clinical Signs

There were no significant differences in tear function between genders except for tear volume which is consistent with a previous study (Sakamoto et al 1993). A higher tear volume in males might be a consequence of a larger acinar area in the lacrimal gland in males (Cornell-Bell et al 1985) and a higher number of hormone receptors on the lacrimal gland in males (Rocha et al 1993, Sullivan et al 1996) which allow more active testosterone-receptor activation to occur as described in 3.6.1.

Previous studies demonstrated significant differences in clinical signs of dry eye between genders, including a lower tear break-up time (Cho & Yap 1993), higher tear evaporation rate (Guillon & Maïssa 2010), higher tear osmolarity (Farris et al 1986)

and lower amount of secreted meibomian lipid (Chew et al 1993) in females. However, there were also studies which reported no gender differences in clinical signs such as NIBUT (Ozdemir & Temizdemir 2009) and meibomian gland assessment score (Schaumberg et al 2011, Viso et al 2012).

The prevalence of meibomian gland disease is higher in Asian (Jie et al 2009, Lekhanont et al 2006, Lin et al 2003, Uchino et al 2006) compared to Caucasian populations (McCarty et al 1998, Schein et al 1997a). Most subjects in the current study are Caucasian (56%) and this may support the "within-normal-values" nature of Marx's line displacement and meibomian gland expressibility. However, the numbers of glands; and capped and patent (expressible) glands were consistent with mild dry eye. Of note, there were no significant differences in meibomian gland assessment between genders in the current study as opposed to a higher prevalence of meibomian gland disease in men (Siak et al 2012).

A significant displacement in Marx's line with androgen as recorded in the current study was a novel finding since a previous study suggested that displacement of mucotaneous junction is not age related (Hykin & Bron 1992). A reduced number of patent meibomian glands with age were recorded in the current study which is consistent with a previous study (Arita et al 2008). In addition, this change might also occur due to the uneven distribution of stressed tissues in the coronel and sagittal planes of lid margin causing narrowing and obliteration of the orifices (Hykin & Bron 1992). A higher tear osmolarity was associated with age in the current study as previously proposed (Mathers et al 1998, Smith et al 2007). However, there were no significant associations between other clinical signs and age as previously demonstrated (Schein et al 1997b).

3.7 Study Limitations and Considerations

Age was believed to confound the significant associations between hormone concentrations and ocular surface sensitivity, symptoms and signs. In addition, the ocular surface sensitivity might have received the impact of diurnal variability (Stapleton et al 2004) which was measured between 8 A.M to 8 P.M.

The convenience sample consisted of normal to mild dry eye subjects who were categorised based on the Women's Health Study questionnaire. However, since the categorization was based on the frequency of symptoms and previous diagnoses, there is a possibility of the presence of subjects' uncertainty about the symptoms frequency (for instance between sometimes and often) and the hidden undiagnosed dry eye subjects within the cohort, who may have biased the responses of the group as a whole. The presence of such subjects would mean that the range of response in the normal and mild group may have been greater than it should have been. This increased variability may therefore have acted to reduce the discriminative ability of the study as a whole.

Circadian variation was reported for testosterone measured from blood collected after noon (Yen & Jaffe 1991) and blood from patients who were fasting is preferable to minimise the change in hormone levels associated with eating (Panico et al 1990). In the current study, subjects were not fasting prior to the blood collection which occurred between 9 A.M to 5 P.M. In almost 50% of the subjects, venous blood collection was performed after 24 to 76 hours from time of the visit which might not accurately represent the associations between the sex hormone concentration and the study variables.

The plasma level of DHEA-S in postmenopausal women decreases over time consistent with aging, with 25% decrease in 5 years of storage in liquid nitrogen freezers (Hankinson et al 1995). Although such effect has not been shown in normal population, the DHEA-S concentration in the current study might have also been affected since the plasma samples were stored for almost a year. Apart from DHEA-S, the reproducibility of oestradiol, testosterone and SHBG were also investigated but none of them were significantly affected by the duration of storage (Hankinson et al 1995). Sex hormone concentration reproducibility study should hence be carried out

on a known plasma sample to confirm if similar decrease in the hormone concentration occurred in the current study.

3.8 Conclusion

Sex hormones were not consistent predictors of symptoms in a normal-to-mild dry eye population consisting of males, menstruating and postmenopausal females who were included even if they were on hormone treatment. It is important to adjust for age and gender when considering the effects of sex hormone levels on dry eye since aging affects sex hormone concentration and several dry eye clinical signs, while gender affects tear volume significantly. Clinicians may need to consider the menstrual status or patients' consumptions of hormonal medication when performing dry eye clinical tests on females. The next chapter will establish whether sex hormones affect symptoms and signs of dry eye in a more homogenous population with age and gender controlled of postmenopausal women with symptomatic dry eye.

CHAPTER 4

The Effects of Circulating Sex Hormone Levels on Ocular Surface Sensitivity and Dry Eye Symptoms and Signs in Postmenopausal Women with Dry Eye

4.1 Introduction

The effects of sex hormones on symptoms and signs of dry eye were demonstrated in a normal-to-mild dry eye population in Chapter 3. The relationship between sex hormone and dry eye is further investigated in this chapter in a homogenous, age and gender controlled population of postmenopausal women with symptomatic dry eye. Higher circulating levels of oestradiol but lower circulating levels of androgens in this symptomatic population compared to the normal population are hypothesised to result in higher symptoms, lower ocular surface sensitivity and greater dry eye signs.

Studies of hormones and dry eye in postmenopausal women have resulted in conflicting findings. A large scale epidemiological study in the US has demonstrated a higher rate of dry eye disease in postmenopausal women using either oral oestrogen or oestrogen and progesterone supplements compared with postmenopausal women not taking supplements (Schaumberg et al 2001). However, circulating hormones were not measured to provide a direct link between hormone levels and symptom reporting.

This finding is broadly consistent with a clinical study in non-dry eye postmenopausal women demonstrating that better tear function is associated with higher levels of circulating androgen and lower oestradiol (Mathers et al 1998). Similarly, dry eye in Sjögren syndrome, which predominantly affects menopausal women, appears to be underpinned by low androgen levels (Sullivan et al 2003). On the basis of these studies, it would appear that high oestrogen and low testosterone are likely to be associated with dry eye in postmenopausal women. However, such associations have not been reported in the postmenopausal women with non-Sjögren dry eye.

Interestingly, low circulating levels of both oestrogen and testosterone have been recently reported with increased symptoms (OSDI) and sign (high osmolarity) of dry eye in postmenopausal women without hormones supplementation (Gagliano et al 2014, Scuderi et al 2012). However, there are no reports of the relationships between the hormone concentration and other important dry eye clinical indicators such as corneal and conjunctival staining, tear volume and tear break-up time. Another study showed no association between oestrogen level and ocular symptoms in postmenopausal women with Sjögren syndrome without supplementation (Taiym et al 2004). The two studies (Gagliano et al 2014, Scuderi et al 2012) reporting a significant association between symptoms and tear osmolarity with the circulating oestrogen and testosterone, used free testosterone as a measure of the androgen pool. This analyte might not be the most accurate marker in this population since the main source of androgen in postmenopausal women is through intracrinology(Labrie et al 2003).A feasible method of measuring androgen levels in this population is to identify the levels of its transformed metabolites and glucuronides such as 3α-diol G and androgen conjugated metabolites (ADTG), which diffuse into the general blood circulation and are the only route of elimination for androgens (Labrie et al 2006). 3α-diol G and ADTG in the circulation represent the level of testosterone at the peripheral site (Labrie et al 2006, Labrie et al 2003).

Apart from the androgen metabolites, DHEA and DHEA-S are also the important androgen precursors in the intracrinology process (Labrie 2010).The concentration of DHEA-S is more easily measured than DHEA since the former is more abundant in the circulation with 300 to 500 times higher concentration (Gordon et al 1990). Therefore ADT-G or 3α-diol G and DHEA-S should be considered when examining the relationship between sex hormones and dry eye in postmenopausal women.

Psychosocial factors might also confound the relationship between hormone levels and symptoms of dry eye. Due to the complexity of changes in life during menopause, psychological and social changes should be specifically considered when examining ocular symptoms in postmenopausal women. The Menopause-Specific Quality of Life (MENQOL) questionnaire is able to elicit the effect of the psychological and social factors which includes the domains of vasomotor, psychosocial, physical and sexual symptoms (Hilditch et al 1996)on dry eye. Self-esteem items were included in the

psychosocial domain. These items, as measured with Rosenberg's scale (Rosenberg 1965), were significantly associated with asthenopia(blurred vision, ocular soreness, itching of the eyes, blinking, heaviness of the eyes, and double vision)in a normal population (Mocci et al 2001). Dry eye symptoms have also been associated with systemic symptoms such as stiffness of the head and shoulder and pain in joints (Shimmura et al 1999). These physical symptoms are among the items queried in the physical domains of the MENQOL (aching in muscle and joints; and back of neck and head). In addition, among the questions addressed in the sexual domain is vaginal dryness which was positively associated with eye dryness (Stadberg et al 2000). However, there are no reports on associations between the vasomotor domains of MENQOL and ocular symptoms in the literature. Furthermore, the relationship between systemic symptoms and dry eye has not been fully investigated in postmenopausal women with dry eye. Hence the associations between the domains of MENQOL with dry eye symptoms and signs are examined in this study.

This chapter aims to investigate the relationships between circulating sex hormones and dry eye in postmenopausal women with dry eye. The study also aimed to identify the potential predictors of symptoms from a panel of variables which included sex hormone concentrations, the ratio of sex hormones, systemic symptoms, ocular surface sensitivity and dry eye clinical signs. Higher circulating levels of oestradiol but lower circulating levels of androgens in this symptomatic population compared to the normal population are hypothesised to result in higher symptoms, lower ocular surface sensitivity and greater dry eye signs. Non inclusion of progesterone hormone in this study was due to the lack of significant associations between the hormone and the variables in the study described in Chapter 3.

4.2 Aims

a) To investigate the associations between circulating concentrations of oestradiol, DHEA-S, 3α-diol G, the ratio of oestradiol to 3α-diol G and dry eye symptoms, systemic symptoms (MENQOL domain scores),ocular surface sensitivity and clinical signs of dry eye in postmenopausal women with dry eye.
b) To identify the predictors of ocular surface symptoms from a panel of variables which include sex hormone concentrations, the ratio of oestradiol to 3α-diol G, systemic symptoms, ocular surface sensitivity and clinical signs of dry eye in postmenopausal women with dry eye.

4.3 Hypotheses

a) A higher circulating level of oestradiol and the ratio of oestradiol to 3α-diol G are associated with
    i. higher ocular surface symptoms
    ii. higher systemic symptoms
    iii. lower ocular surface sensitivity
    iv. greater dry eye signs
b) A higher circulating level of 3α-diol G and DHEA-S is associated with
    i. lower ocular surface symptoms
    ii. lower systemic symptoms
    iii. higher ocular surface sensitivity
    iv. fewer dry eye signs
c) Ocular symptoms are positively associated with systemic symptoms scores

4.4 Method

This cross-sectional study was conducted at the School of Optometry & Vision Science (SOVS). Subjects were enrolled for a two-hour visit between 9am to 4pm. Ethics approval was obtained from Human Research Ethics Committee (HREC12087) at the University of New South Wales (UNSW), Sydney, Australia and followed the tenets of the Declaration of Helsinki. Signed informed consent was obtained from each

participant prior to enrolment in the study (Appendix 3). Subjects were recruited via advertisement in community newspapers; senior societies' and senior clubs' newspapers, notice boards and websites; database of subjects of previous studies conducted at SOVS; generic emails circulated to the SOVS and UNSW staff; invitation letters to the above 50 year old female patients of the UNSW Optometry clinic; posters and flyers placed around the campus of the University of New South Wales.

Subjects were recruited through advertisements and announcements in community newspapers; senior societies' and senior clubs' newspapers, notice boards and websites; database of subjects of previous studies conducted at SOVS; generic emails circulated to the SOVS and UNSW staff; invitation letters to the above 50 year old female patients of the UNSW Optometry clinic; posters and flyers placed around the campus of the University of New South Wales. Telephone or face to face screening interviews were carried out on 200 subjects who responded to the study recruitment invitation.

4.4.1 **Subjects**

The sample size was based on a previous publication reporting an association between circulating hormone levels (oestradiol) and tear osmolarity in postmenopausal women (Mathers et al 1998). Tear osmolarity was chosen as it was among the tests conducted in the current study and reported as a gold standard for the diagnosis of dry eye(Lemp et al 2011). Based on a rho value of -0.5, power of 90% and alpha = 0.01**,** sample size calculation indicated that 45 subjects were required to demonstrate an association between circulating oestradiol and tear osmolarity. To understand the mechanism behind the relationship between sex hormones and dry eye without the possible interference of auto antibodies and autoimmune connective tissue related disease (Caffery et al 2010a, Vitali et al 2002), only postmenopausal women with non-Sjögren's dry eye were recruited.

Inclusion and Exclusion Criteria

Inclusion criteria

- Female gender

- Age 50 years and above
- Permanent menstrual cessation for at least 1 year
- Diagnosis of dry eye based on Women's Health Study criteria (Schaumberg et al 2001)

- This dry eye classification questionnaire consists of three questions on the frequency of symptoms of dryness and irritation, on a 1 - 4 scale where 4 represents constant, 3 represents often, 2 represents sometimes and 1 represents never. The questionnaire also includes an item on previous history of clinically diagnosed dry eye. Subjects with responses of 'constant' and 'often' to dryness and irritation, or who had been previously diagnosed with dry eye were classified as having dry eye (Schaumberg et al 2001).

Exclusion criteria

- Sjögren's syndrome based on European Classification of Sjögren's syndrome (Vitali et al 2002) based on all of the following criteria
  i. shows positive response to at least one of the ocular symptoms questions,
  a) Have you had daily, persistent, troublesome dry eyes for more than 3 months?
  b) Do you have a recurrent sensation of sand or gravel in the eyes?
  c) Do you use tear substitutes more than 3 times a day?
  ii. shows positive response to at least one of the oral symptoms questions
  a) Have you had a daily feeling of dry mouth for more than 3 months?
  b) Have you had recurrently or persistently swollen salivary glands as an adult?
  c) Do you frequently drink liquids to aid in swallowing dry food?
  iii. less than 5 mm of wetted Schirmer test strip in 5 minutes

  Subjects who show positive responses to i and ii and iii are considered as potential Sjögren's syndrome patient and is confirmed with

  iv. the presence of antibodies to Ro(SSA) or La(SSB) antigens, or both in the serum
- History of corneal or refractive surgery
- History of hormone therapy within the past 12 months

- History of hysterectomy and/or oopherectomy
- Prior diagnosis of infectious disease transmittable by blood (eg HIV/AIDS, Hepatitis)
- Eye surgery within the past 6 months immediately prior to enrolment for this study
- Use of antidepressant medication if first started within the past 12 months
- Subjects with ocular or systemic disease and/or associated treatment deemed likely to significantly impact on the ocular surface
- Use of systemic or topical medication likely to significantly affect ocular physiology (eg anti-acne medications such as Roaccutane, corticosteroid or immunosuppressant medications such as Hydrocortisone, Prednisolone, and antihistamine medications)

Use of systemic or topical medication whose effect may be reduced by oestrogen therapy (eg anticonvulsants, meprobamate, phenylbutazone, griseofulvin and rifampicin)

- Subjects with systemic disease and/or associated treatment which is likely to be significantly affected by the treatment product for example:
    - Severe hepatic and renal disease
    - Known or suspected carcinoma of the breast, endometrium or other oestrogen dependent neoplasia
    - High blood pressure

4.4.2 **Procedures**

Serum hormone concentrations; systemic symptoms; ocular symptoms; ocular surface sensitivity and integrity; tear function; and meibomian gland assessments were evaluated. Procedures are as described in 3.4.2, except for the serum harvesting method and the additional questionnaires [Menopause-Specific Quality of Life (MENQOL) (Hilditch et al 1996)]. The Schirmer test was also included in this study. Variables were measured in the order described below.

4.4.2.1 Questionnaires

Self-administered questionnaires were presented to study subjects in electronic format as described below.

a) Ocular Symptoms
   i. Ocular Comfort Index [(OCI) (Johnson & Murphy 2007)] Appendix B. The intensity and frequency of dryness were also analysed
   ii. Ocular Surface Disease Index [(OSDI) Allergan Inc, Irvine, California USA 2004] Appendix C
b) Systemic Symptoms

Menopause-Specific Quality of Life (MENQOL) (Hilditch et al 1996) assessed the vasomotor, psychosocial, physical and sexual domains of subjects (Appendix H). There is no overall score that can be obtained from this questionnaire since the relative contribution of each domain to an overall score is unknown (Hilditch et al 1996). The average score of each domain was recorded as1-8 where 8represents the greatest discomfort (extremely bothered).Menopause-Specific Quality of Life (MENQOL) (Hilditch et al 1996) assessed the vasomotor, psychosocial, physical and sexual domains of subjects (Appendix H). There is no overall score that can be obtained from this questionnaire since the relative contribution of each domain to an overall score is unknown (Hilditch et al 1996). The average score of each domain was recorded as 1 - 8 where 8 represents the greatest discomfort (extremely bothered). However in this thesis, the score was based on a scale from 1 to 7 where 7 represents the greatest discomfort, due to the accidental removal of the first score of “not bothered” and instead the scoring started with the score of “yes”, which resulted in reduced scores.

4.4.2.2 Tear function assessments

Tear function assessments were performed bilaterally in the following order with the right eye tested first as described in 3.4.2.2.

i. Tear osmolarity (Ocusense TearLab Osmolarity System, TearLab corporation, CA, US)
ii. Non-invasive Tear Break-Up Time (Keeler Tearscope-plus^{R}, Keeler, Windsor, UK)
iii. Tear volume (Phenol Red Thread, PRT ZONE-QUICK, Showa Yakuhin Kako Co., Ltd, Japan)

4.4.2.3 Ocular Surface Sensitivity

Corneal and conjunctival sensitivity were measured on the right eye with the Cochet-Bonnet aesthesiometer (Luneau Ophthalmologie, France) using 0.08 mm thread as described in 3.4.2.3.

4.4.2.4 Tear Volume (Schirmer Test)

A Schirmer strip (Alcon Laboratories, Sigma Pharmaceuticals, Iowa, USA) was placed in the lower temporal fornix of the left eye and left in place for 5 minutes. The patient was told to blink normally. After 5 minutes, the strip was removed and the wet length was recorded in millimetres.

4.4.2.5 Ocular surface integrity assessments

Assessments were performed bilaterally as described in 3.4.2.4

4.4.2.6 Meibomian gland and lid margin assessments

Assessments were performed bilaterally as described in 3.4.2.5

4.4.2.7 Serum Hormone Concentration

Venous Blood Collection, Processing and Storage

Venous blood was collected on the same day as the other study variables. Nine ml of venous blood were drawn into a 9 ml serum separating tube (VACUETTE® Serum Separator tubes, Greiner Bio-one, Austria).

The serum harvesting method was based on Tuck et al (2008)

i. The blood was allowed to clot for 30 minutes at room temperature

ii. The blood was centrifuged for 15 minutes at 1500g at 4°C

iii. The serum layer above the gel was collected and transferred using a sterile pipette into 5 Eppendorf tubes of 1 ml aliquots

iv. The serum was stored at –80°C for 1 to 9 months prior to analysis

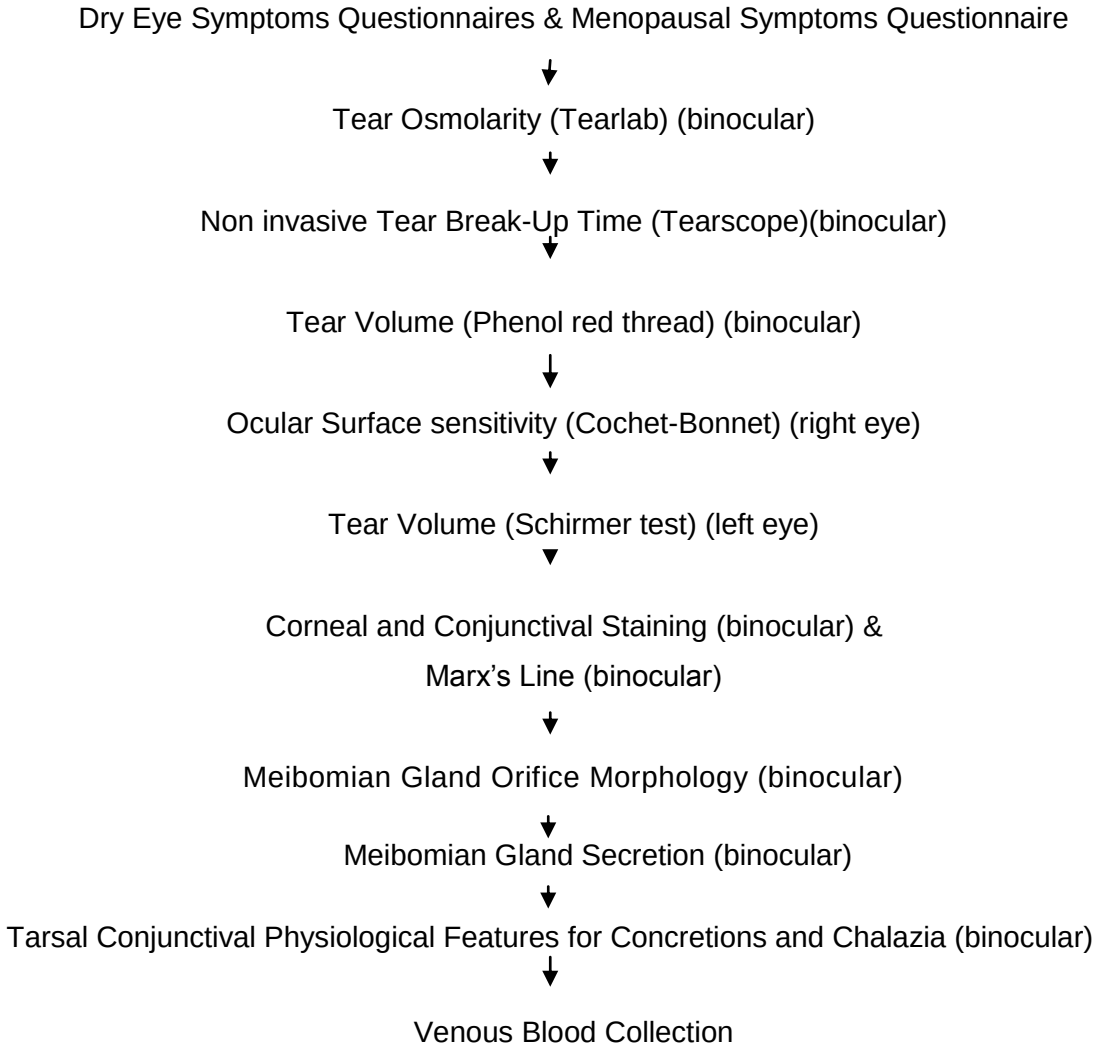
v. Samples were thawed and inverted several times prior to testing

vi. Serum concentration of 3α-diol G, DHEA-S and oestradiol were measured with ELISA kits from DRG International, USA-NJ (2012-13). The measured sex hormones and their limits of detection based on the assay kits(Table 4.1). The procedures for ELISAs was described in 3.4.2.6.1.1

Table 4.1 Sex Hormones (ELISA kit) and Their Limits of Detection

Sex Hormones	Limits of detection
Oestradiol (EIA 4399)	1.4 pg/mL
3α-diolG(EIA-4192)	0.25 ng/ml
DHEA-S (EIA 1562)	0.04 µg/ml

4.4.2.8 Flow chart of tests performed on subjects



4.5 Statistical Analysis

Data normality was assessed with using the Kolmogorov-Smirnov test ($p>0.05$). For the univariate analysis, associations between the study variables (ocular symptoms, MENQOL domain scores, serum concentrations of sex hormones, ratio of oestradiol to androgen and clinical signs of dry eye) were assessed with Pearson (parametric) and Spearman (non-parametric) Bivariate Correlation tests ($p<0.05$). For multivariate analysis, determination of independent variables that predict ocular symptoms was performed using a general linear model (section 4.5.1.1 below). Mann-Whitney test and

independent sample t-test were used to compare the sex hormone concentration between postmenopausal women with and without dry eye (p<0.05).

4.5.1.1 Standard Multiple Regression Analysis

Spearman and Pearson Bivariate Correlation tests were used as appropriate to examine associations between various ocular symptom scores and independent variables (sex hormone concentration, MENQOL domain scores, tear volume, tear osmolarity, NIBUT, ocular surface sensitivity and staining, meibomian gland and lid margin assessments). The ocular symptom score chosen as the dependent variable was based on the metric with the highest number of significant associations (p<0.25) from the univariate analysis. The independent variables associated with symptoms at p<0.25 were entered into a general linear model. The final model for significant variables was determined using the method of backward elimination followed by forward entry, and chosen based on optimising the values of the R Square and p value. The independent variables in the final model were retained only if they were significant at $p < 0.05$.

4.6 Results

The study was conducted between 18th July 2012 and 17th April 2013. The age range of the moderate dry eye subjects was 53 -83 (mean 64.8 ± 5.2) years. The age range of years since menopause was 2-35 (12.9 ± 6.6) years. One subject was on thyroid medication and one subject had an ovary removed by surgery. Normality of duration of menopause and study variables is displayed in Table 4.2.

Table 4.2 shows the Kolmogorov-Smirnov test for normality of distribution. Variables with p> 0.05 have a normal distribution. Normally distributed data are indicated in bold.

Table 4.2 Normality of Study Variables with indication of normal data in bold (n=45)

Variables	Kolmogorov-Smirnov p values
Years since menopause	p< 0.001
Sex Hormone Concentration	
Oestradiol (pg/mL)	p< 0.001
5alpha-androstane-3alpha and 17beta-diolglucuronide (3α-diol G) (ng/ml)	p< 0.001
Dehydroepiandrosterone Sulfate (DHEA-S) (µg/ml)	p< 0.001
Oestradiol:3α-diol G	p< 0.001
Ocular Symptoms Scores	
Ocular Surface Disease Index	**0.20**
Ocular Comfort Index	**0.20**
Ocular Comfort Index (OCI) Dryness Intensity	0.01
Ocular Comfort Index (OCI) Dryness Frequency	p< 0.001
MENQOL domain scores	
Psychosocial	p< 0.001
Physical	0.01
Vasomotor	**0.20**
Sexual	**0.09**
Ocular Surface Sensitivity	
Central cornea [(CCS (1/g/mm^2)] (Right Eye)	p< 0.001
Inferior conjunctiva [(ICJS (1/g/mm^2)] (Right Eye)	p< 0.001
Clinical Signs	
Tear osmolarity [(mOsms/L)Worst Eye]	p< 0.001
Tear Volume [PRT (mm)(Average of both eyes)]	**0.20**
Tear Volume [(Schirmer (mm)(Left Eye)]	0.01
Non invasive tear break-up time [NIBUT(s)(Average of both eyes)]	p< 0.001
Corneal Staining[(grade) (Average of both eyes)]	p< 0.001
Conjunctival Staining [(grade)(Average of both eyes)]	**0.13**
Marx's Line[(grade)(Average of both eyes)]	p< 0.001
Vascularity of lower lid margin [(grade)(Average of both eyes)]	p< 0.001
Telangiectasia on lower lid margin [(grade)(Average of both eyes)]	p< 0.001
Meibomian gland expressibility on lower lid margin [(grade)(Average of both eyes)]	p< 0.001
Number of glands on lower lid margins [(number)(Average of both eyes)]	p< 0.001
Number of capped glands on lower and upper lid margins [(number)(Average of both eyes)]	p< 0.001

4.6.1 **Serum Sex Hormone Concentrations**

Oestradiol, DHEA-S and 3α-diolG serum concentrations are shown in Table 4.3. A wider than the normal published range was recorded for oestradiol and 3α-diol G. The maximum concentration of oestradiol and 3α-diol G exceeded the manufacturer's guideline range for normal postmenopausal women (Figures 4.1 and 4.2). The range for DHEA-S level was within normal values.

Table 4.3 Sex Hormone Serum Concentrations (n=45)

Variable	Range (IQR/Median)	Published values in normal postmenopausal women
Oestradiol: pg/mL	1.6–108.1 (9.12/4.6)	11-65*
3α-diol G: ng/ml	0.4-14.4 (1.2/1.5)	0.1-5.9*
DHEA-S: µg/ml	0.1-2.9 (0.3/0.6)	0.1-3.0 (Davison et al 2005)
Oestradiol:3α-diolG	0.8-19.5 (3.1/5)	Not available

***DRG International Inc CA, USA Manual 2006-2011.

Figure 4.1 Oestrogen Serum concentration (n=45)

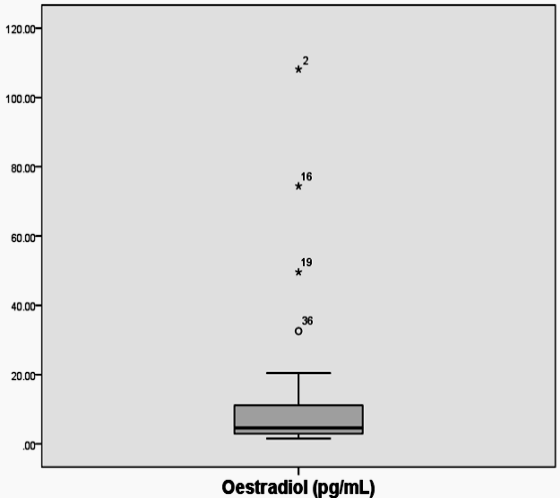
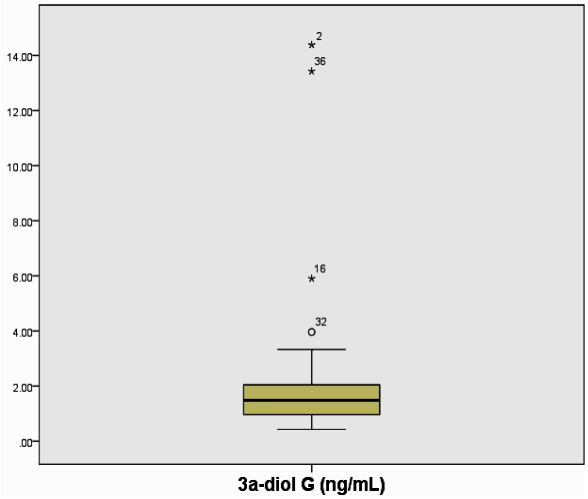


Figure 4.2 3α-diol G Serum concentration (n=45)



Circles represent the outliers who had serum concentration of oestradiol (figure 4.1) and 3α-diol G (figure 4.2) belonging to the respective subjects with the identification number.

4.6.2 **Ocular Symptoms**

Ocular Symptoms

Scores for OSDI and OCI (Table 4.4) are broadly within the expected ranges for subjects with moderate dry eye.

Table 4.4 Ocular Symptoms Scores (n=45)

Ocular Symptoms Questionnaires	Mean ± SD	Published Values in Dry Eye
Ocular Surface Disease Index (OSDI)	27.3 ±18.1	24.9 ±13.9 (postmenopausal women) (Srinivasan et al 2008)
Ocular Comfort Index (OCI)	40.3 ± 8.9	39.6 ± 10.2 (Chao et al 2013). 40 (Evans et al 2009)
Frequency of dryness (OCI)	3.5 ± 1.3	Not available
Intensity of dryness (OCI)	4.3 ±1.6	Not available

4.6.3 **Systemic Symptoms**

Scores for all domain systemic symptoms based on the Menopause-Specific Quality of Life MENQOL are shown in Table 4.5. Mean group scores for the symptoms were within the range of the lowest published values of the MENQOL domain scores in a non-dry eye population.

Table 4.5 Menopause-Specific Quality of Life (MENQOL) Scores (n=45)

Domains	Mean ± SD	Lowest published values (Mean ± SD) (Haines et al 2005)
Vasomotor	2.5 ± 1.2	2.2 ± 1.4
Psychosocial	2.1 ± 1.1	2.4 ± 1.3
Physical	2.8 ± 1.1	2.7±0.6
Sexual	3.2 ± 1.8	2.1 ±1.3

4.6.4 **Ocular Surface Sensitivity**

Ocular surface threshold was lower (higher ocular surface sensitivity) than published in dry eye (Table 4.6). Temporal conjunctival sensitivity was the only recorded sensitivity for conjunctiva (Toker & Asfuroglu 2010). Measurements were conducted in a room with an ambient humidity range of 42-59% and 23-25ºC temperature. Figures 4.3 and 4.4 display the ocular surface sensitivity.

Table 4.6 Ocular Surface Sensitivity Measurements (n=45)

Ocular Surface	Sensitivity [1/g/mm^2]	Threshold (cm)	Published values of threshold (cm) in dry eye
Mean ± SD			
Cornea	3.3 ± 3.3	4.2 ± 1.4	5.2 ± 0.6 (Toker & Asfuroglu 2010)
Conjunctiva	0.5 ± 0.9	1.7 ± 1.6 (Inferior)	1.9 ± 0.7 (Temporal) (Toker & Asfuroglu 2010)

Figure 4.3 Corneal Sensitivity Distribution (n=45)

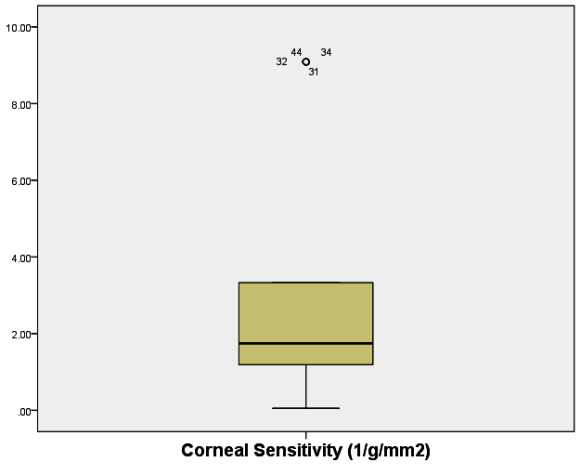
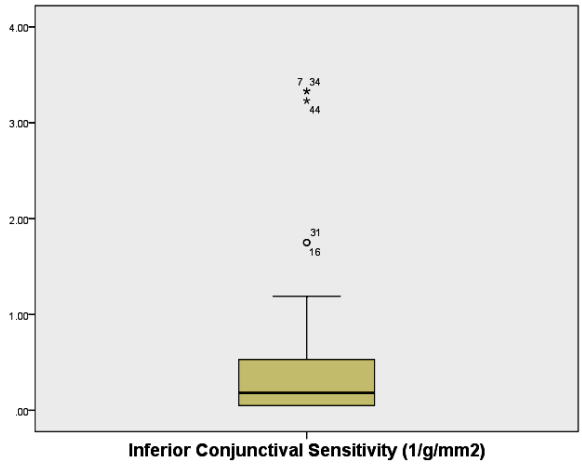


Figure 4.4 Conjunctival Sensitivity Distribution (n=45)



Circles represent the outliers who had corneal sensitivity in figure 4.3 and inferior conjunctival sensitivity in figure 4.4 belonging to the respective subjects with the identification number.

4.6.5 **Dry Eye Clinical Signs**

Table 4.7 shows the clinical signs relative to previously published values in a normal population and in postmenopausal women with dry. Tear osmolarity, tear volume (measured with PRT and Schirmer tests), NIBUT, Marx's line score and the number of meibomian glands and telangiectasia of the current study were within normal values. Only ocular surface staining, vascularity, meibomian gland expressibility (secretion quality and expressibility) and number of capped glands were consistent with meibomian gland disease. There were 5 subjects for whom NIBUT could not be recorded. Data in these cases were recorded as average time to first blink.

Table 4.7 The Dry Eye Clinical Signs Scores and their Published Values (n=45)

Variable	Mean ± SD [Range/IQR]	Published values
Tear Osmolarity (mOsms/L)	306.6±13.8	315.5 ±10.4 in mild to moderated
		308 in Non-Dry Eye (Lemp et al 2011)
Phenol Red Thread (PRT) (mm)	24.5 ± 4.7	16.3± 5.6 in DE postmenopausal women (Srinivasan et al 2008)
		19.7± 5.9 in NDE (Doughty et al 2007)
Schirmer (mm)	10.7± 7.9	1.4 ± 1.3 in DE postmenopausal women(Scuderi et al 2012)
		<20 in moderate DE (Lemp et al 2011)
Non-invasive tear break-up time NIBUT (sec)	10.8 ± 4.3	5.3 ±1.7 in DE postmenopausal women (Srinivasan et al 2008)
		<10 in moderate DE (Lemp et al 2011)
Corneal Staining (Grade)	0-3/1.5/0.5	> 0 in moderate DE (Lemp et al 2011)
Conjunctival Staining (Grade)	0-6/3.1/2.0	> 1 in moderate DE (Lemp et al 2011)
Marx's Line [(grade)	1.2 ±1.8	5.9 ±2.0 in MGD (Yamaguchi et al 2006)
Vascularity of lower lid margin (grade)	0-2/1.0/0.5	≥2 in MGD (Bron et al 1991)
Telangiectasia on lower lid margin (grade)	0-1.5/0.4/0	≥2 in MGD (Bron et al 1991)
Meibomian gland Expressibility of lower lid margin (grade)	0-6/2.8/0	>2 in MGD (Bron et al 1991)
Number of Meibomian glands &Capped glands on lower and upper lid margins	2-8/1/5 &0-13/2.8/0	6-7 in normal adult (Hykin & Bron 1992)/

DE: Dry eye
MGD: Meibomian gland disease

4.6.6 **Univariate Analysis**

Associations in between sex hormone levels, ratio of oestradiol to androgen and ocular symptoms; systemic symptoms; ocular surface sensitivity and clinical signs are presented in appendices T.

4.6.6.1 Association between Sex Hormone Concentrations and Study Variables

Oestradiol concentration and the ratio of oestradiol to 3α-diol G were positively associated with corneal staining (Figures 4.5 and 4.6). However, there were no relationships between hormones and other signs or symptoms of dry eye, systemic symptoms and years since menopause.

Figure 4.5 Association between oestradiol and corneal staining (n=45)

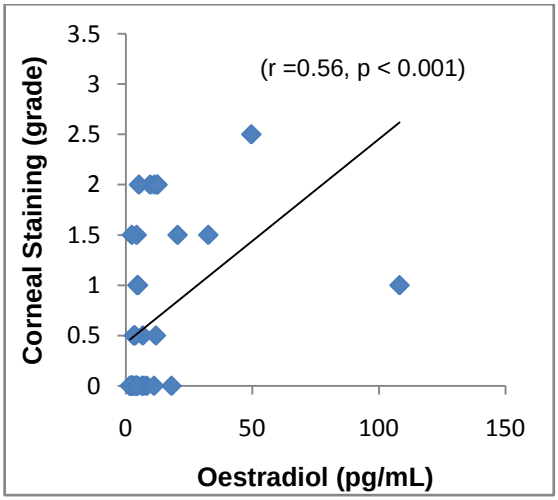
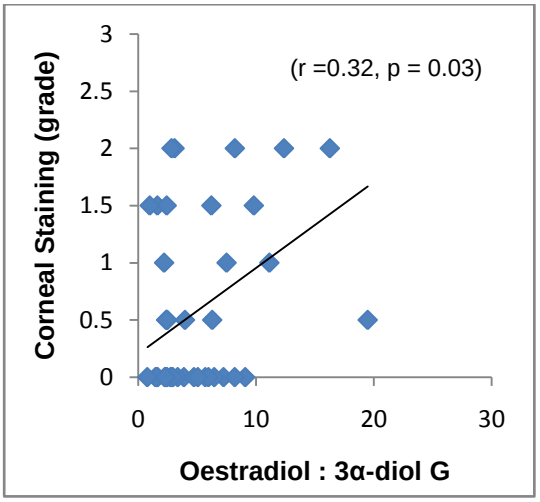


Figure 4.6 Association between the ratio of oestradiol to 3α-diol G and corneal staining (n=45)



4.6.7 **Multivariate Analysis**

OCI frequency of dryness was chosen as the outcome measure for multivariate analysis because it was the metric with the highest number of significant associations (p<0.25) from univariate analysis (appendix T). Significant univariate relationships were observed between OCI frequency of dryness and oestradiol, meibomian gland expressibility, lid margin vascularity and number of capped glands. These were used in

the development of the model. The model explained 33.5% of the variance in the OCI frequency of dryness and was statistically significant ($p<0.005$) (Table 4.8). The equation generated for OCI frequency of dryness is = 0.37 (meibomian gland expressibility) - 0.93 (vascularity) + 4.46 (bolded in Table 4.8). With higher meibomian gland expressibility and lower vascularity of lid margin, the OCI frequency of dryness scale was expected to be higher (more frequent symptoms) after controlling for the other variables in the model.

Table 4.8 The Multivariate analysis for Ocular Surface Symptoms (n=45).

Dependent variable	**OCI Dryness frequency**	
Significant univariate relationships with	Oestradiol	
	Number of capped glands	
	meibomian gland expressibility	
	Vascularity of the lid margin	
	Unstandardized Coefficients β	**p values**
Independent variables in the final model	4.46 Constant	0.00
	-0.02 Oestradiol	0.16
	-0.05 number of capped glands	0.48
	0.37 meibomian gland expressibility	**0.01**
	-0.93 vascularity of the lid margin	**0.02**
R^2%	33.5	
p value	0.002	
Predictor/s	0.37 meibomian gland expressibility -0.93 vascularity of lid margin	
Equation	OCI Dryness frequency = 0.37 meibomian gland expressibility -0.93 vascularity of lid margin + 4.46	

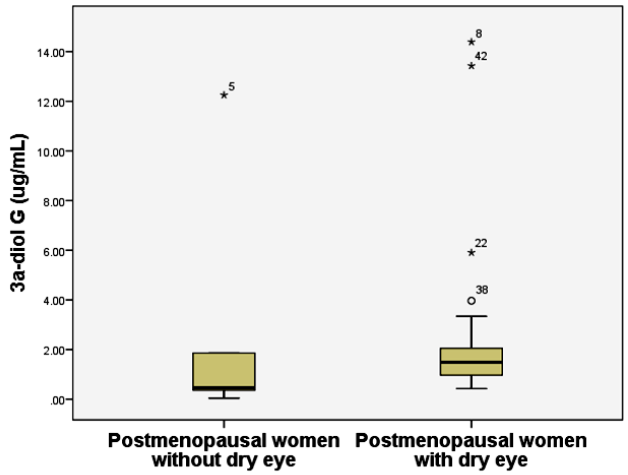
Unstandardized Coefficients column with significant p values ($p<0.05$) are bolded

4.6.8 **Comparison of Circulating Sex Hormone Concentrations between Postmenopausal Women with and without Dry Eye**

The concentration of oestradiol, 3α-diol G, DHEA-S and ratio of oestradiol to 3α-diol G measured in plasma in postmenopausal women without dry eye (data of six subjects who were not using Hormone Replacement Therapy from Chapter 3) was compared with serum concentration of the same hormones in postmenopausal women with dry eye in the current chapter. There were no significant differences in the hormones

concentration between the two groups. Figures 4.7 to 4.10 displayed the comparison of the sex hormones concentrations between the groups.

Figure 4.7 The 3α-diol G concentration in postmenopausal women with (n=45) and without (n=6) dry eye (p = 0.14)..

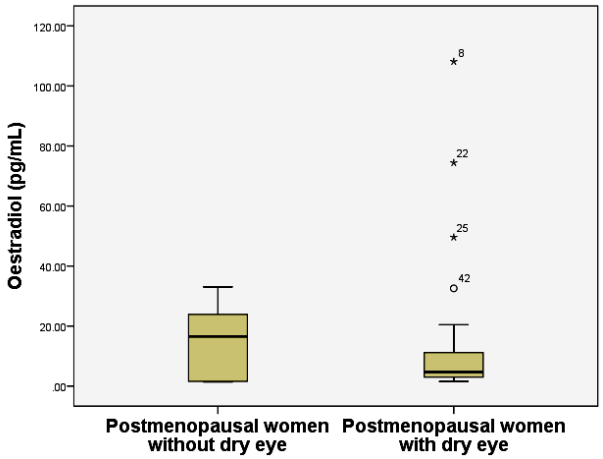


Circles represent the outliers of the serum concentration 3 α-diol G belonging to the respective subjects with the identification number

Figure 4.8 The DHEA-S concentration in postmenopausal women with (n=45) and without (n=6) dry eye (p =0.48).

Circles represent the outliers of the serum concentration of DHEA-S belonging to the respective subjects with the identification number.

Figure 4.9 The oestradiol level in postmenopausal women with (n=45) and without (n=6) dry eye (p = 0.56).



Circles represent the outliers of the serum concentration of oestradiol belonging to the respective subjects with the identification number.

Figure 4.10 The ratio of oestradiol to 3α-diol G in postmenopausal women with (n=45) and without (n=6) dry eye (p = 0.06).

Circles represent the outliers of the plasma concentration of 3α-diol G belonging to the respective subjects with the identification number.

4.7 Discussion

4.7.1 General Findings

There were significant univariate positive associations between the worsening of corneal staining and both oestradiol concentration and the ratio of oestradiol to 3α-diol G. Oestradiol was among the factors in the final model of the regression analysis but was not a consistent predictor of symptoms, which instead were affected by meibomian gland expressibility and the vascularity of the lid margin.

Based on the comparison between the sex hormone concentrations in the current study and literature, the range of the oestradiol concentration in the postmenopausal women with dry eye was lower than postmenopausal women without dry eye, while the concentration of the 3α-diol G and DHEA-S were within normal range. The occasional outliers observed in the oestradiol and 3α-diol G concentrations did not materially change the study findings. In addition there were neither diseases nor conditions that might be related to hormonal variation recorded in the data, suggesting that there was nothing unusual in the participants in this respect. There were significant univariate associations between the worsening of corneal staining and both oestradiol concentration and the ratio of oestradiol to 3α-diol G. Oestradiol was among the factors in the final model of the regression analysis but was not a consistent predictor of symptoms, which instead were affected by meibomian gland expressibility and the vascularity of the lid margin.

Although the subjects were categorised as having moderate dry eye, corneal sensitivity was higher than published in the dry eye subjects while tear osmolarity, tear volume (measured with PRT and Schirmer tests), NIBUT, Marx's line score; and the number of meibomian glands and telangiectasia were within normal values. This condition is consistent with the frequent findings of no associations between symptoms and signs of dry eye (Nichols et al 2004, Sullivan et al 2014). Only ocular surface staining, vascularity, meibomian gland expressibility (secretion quality and gland expression); and number of capped glands were consistent with the values in meibomian gland disease. Based on these findings and the multivariate analysis outcome, more

emphasis should be given to meibomian gland dysfunction as a cause of dry eye symptoms in postmenopausal women.

4.7.2 **Ocular Symptoms**

Circulating sex hormone concentrations were not associated with ocular symptoms in a dry eye population. In the current study, the subjects were categorised as moderate dry eye based on the OSDI score as previously demonstrated in postmenopausal women (Srinivasan et al 2008). The subjects' oestradiol concentration range in the current chapter was lower, instead of higher than the expected oestradiol range in postmenopausal women which may have led to the lack of expected significant associations. This might be caused by the limitations of the ELISA kits used where the results are not standardised in different populations (Rosner et al 2007). In addition, the concentrations of 3α-diol G and DHEA-S were within normal range, instead of lower as predicted in postmenopausal women with dry eye. This normal range of androgens might still be sufficient to regulate the lacrimal and meibomian glands and therefore result in the lack of significant associations between the androgens and dry eye and systemic symptoms.

The contribution of androgen produced by the postmenopausal ovary to the circulating pool of androgen is controversial. Among oophorectomised women, testosterone levels were not affected by age and were 40-50% lower than those in intact women throughout the 50-89 year age range (Fogle et al 2007, Laughlin et al 2000).However the enzymes for androgen biosynthesis were either absent or present in very low amounts in postmenopausal ovary (Couzinet et al 1989). In the present study, the mean of years since menopause was relatively high at 12.9 ± 6.6 years. However, years since menopause was not among the predictors of sex hormone concentration in postmenopausal women (Cauley et al 1989) and the, number of years since menopause was not associated with symptoms in the current study. Therefore it cannot be concluded that the normal range of 3α-diol G and DHEA-S concentration were due to the contribution of androgen by the ovaries.

The meibomian gland expressibility (secretion quality and glands expression) and the vascularity of lid margin were significant predictors of ocular symptoms. The pressure exerted on the meibomian glands and the features of the glands secretion were graded in the assessment of meibomian gland expressibility as described in Section 3.4.2.3.4. Meibomian gland secretion limits evaporative tear loss, provides a barrier function at the lid margin, supplies lubrication during blinking, and maintains an optically smooth ocular surface (Nichols et al 2011). Compromised meibomian gland secretion might thus lead to an increase in tear evaporation rate and hence dry eye.

Vascularity of the lid margin was demonstrated as an important index for gland dropout examination of the meibomian gland (Yamada et al 2005) and is part of the lid margin abnormality score proposed as Diagnostic Criteria for Obstructive Meibomian Gland Dysfunction (Arita et al 2009). However, higher vascularity of the lid margin was associated with reduced symptoms in the current study. Meibomian gland secretion has a melting point range of 32° to 40°C (Olson et al 2003).An increase in the lid margin vascularisation is speculated to relieve the unwanted heat (Nagymihályi et al 2004) to maintain the melting point range, so that the meibomian gland secretion is normally liquid at body temperature (37°C). Therefore increase in the lid margin vascularisation might be able to maintain the meibomian glands temperature and hence secretion which results in less meibomian gland dysfunction and symptoms as revealed by the multivariate analysis.

4.7.3 **Systemic Symptoms**

There were no significant associations between Menopause-Specific Quality of Life (MENQOL) domains and ocular symptoms. Such lack of associations might be due to the mildness of the systemic symptoms (Haines et al 2005) moderate dry eye diagnosis as well as the positive acceptance of the deterioration in physical health (Smeeth & Iliffe 1998) and possibly ocular symptom with age, among the study subjects. This population may have had the impression that these deteriorations were expected in aging process and those in the cohort were not actually “bothered” by the symptoms. The lowest published values of the MENQOL domain scores were compared with the values obtained from the current study to justify the severity of symptoms, since there is no published severity segregation on MENQOL domain

scores. The MENQOL instrument application should be performed cautiously since different scores were shown among different ethnic groups (Haines et al 2005). Although lower scores were obtained, since a lower scoring system was used, this finding may pave the way to investigate the association between the systemic symptoms and dry eye.

4.7.4 **Ocular Surface Sensitivity**

Ocular surface sensitivity was not affected by sex hormones in the dry eye population in which the corneal sensitivity was unexpectedly higher than the normal range.

4.7.5 **Clinical Signs**

A significant association was demonstrated between a higher level of oestrogen and greater corneal staining in the current study. Corneal staining is used to detect disruption of the epithelial cells, as fluorescein dye penetrates the corneal epithelium at points of loose adhesion between cells (Jalbert et al 1999, Morgan & Maldonado-Codina 2009). Oestrogen promotes gene expressibility of inflammatory cytokines and matrix metalloproteinases (MMPs) at the respective oestrogen receptors on the cornea (Suzuki et al 2009). This may lead to corneal surface damage (Feenstra & Tseng 1992) and hence compromises surface integrity that results in greater corneal staining. Corneal staining is used to detect disruption of the epithelial cells, as fluorescein dye penetrates the corneal epithelium at points of loose adhesion between cells (Jalbert et al 1999, Morgan & Maldonado-Codina 2009). Apart from epithelial cells, fluorescein may also penetrate the individual cells of cornea (Wilson et al 1995).

In contrast, a normal range of androgen might still be sufficient to regulate the lacrimal and meibomian glands and may therefore have resulted in the lack of significant associations between circulating androgens and dry eye signs. Signs such as vascularity, meibomian gland expressibility and number of capped glands were consistent with meibomian gland disease. The range of lid margin vascularity and physiological characteristics of the orifices of the meibomian glands were also consistent with meibomian gland disease in a previous intervention study on postmenopausal women with climacteric symptoms (Kuscu et al 2003). A significant

improvement in meibomian gland inflammation was demonstrated with a combination of oestrogen and progesterone treatment, which allowed the impact of sex hormones on the meibomian gland to be observed.

4.8 General Discussion

There was no association between tear osmolarity and hormone levels in the moderate dry eye subjects in the present study, in contrast to the report involving postmenopausal women with severe dry eye (Gagliano et al 2014, Scuderi et al 2012). Subjects with more severe dry eye demonstrated a higher score of tear osmolarity which might have impacted the association between this sign and sex hormone concentrations. Of note, in one of these studies, the lack of significant associations between oestradiol and total testosterone and conjunctival staining, tear volume and tear break-up time was also reported (Gagliano et al 2014). Such lack of significant findings was possibly due to the inappropriate usage of total testosterone as a study variable in the association tests with the respective clinical signs. Total testosterone might not detect the actual androgenic activity in postmenopausal women as the recommended androgen metabolites would, as described in 1.4.1.

4.8.1 **Comparison between dry eye and non dry eye**

The positive effect of the androgen level in improving symptoms and signs was described in Chapter 3. However, there were no significant differences in oestradiol, DHEA-S, 3α-diolG and the ratio of oestradiol to 3α-diol G concentrations between the postmenopausal women with (current study) and without (subjects from the study in chapter 3) dry eye. Although the study was not sufficiently powered to investigate this comparison, the mean concentration of oestradiol, 3α-diol G and the ratio of oestradiol to 3α-diol G were lower in the dry eye group. Lower oestradiol concentration was previously demonstrated in the dry eye than in non-dry eye postmenopausal women (Gagliano et al 2014). In the current study, the ratio of oestradiol to androgen was almost significantly different between these two groups, proposing dry eye as the effect of imbalanced hormone concentrations. The unwanted effects of testosterone are actually caused by the change in the oestradiol level relative to testosterone level (oestradiol/testosterone ratio) (Murphy et al 2000, Rohr 2002). A larger sample size in

future studies may reveal the expected significant difference in the levels of oestradiol, 3α-diol G and DHEA-S between dry eye and non-dry eye females.

4.9 Study Considerations

Serum concentration of sex hormones in postmenopausal women may be affected by obesity, cigarette smoking, alcohol consumption, environmental and lifestyle (Cauley et al 1989) which were not taken into account in the current study.

As explained in Section 3.7, the concentration of sex hormones might be affected by the time of the day when sample was collected (Panico et al 1990, Yen & Jaffe 1991). In the current study, non-fasting blood was collected between 9 A.M to 5 P.M.

The statistical power for this study was exceptionally high at 90%. However, the lack of significant associations between hormone levels and dry eye symptoms and signs could be due to the less severe systemic symptoms and dry eye disease; and lower oestradiol concentration than in expected dry eye population.

4.10 Conclusion

Sex hormones were not consistent predictors of symptoms in a group of postmenopausal women with symptomatic dry eye where symptoms were instead influenced by meibomian gland expressibility and vascularisation of lid margin. Due to the mildness of systemic symptoms, they were neither affected by the circulating sex hormones nor associated with ocular symptoms. The lower oestradiol concentration and within-normal androgens concentration were not in agreement with the study hypotheses. The severity of disease may affect the associations and should be considered in future studies.

The next chapter will explore whether sex hormone treatment affects symptoms and signs of dry eye in the post-menopausal women population with symptomatic dry eye.

CHAPTER 5

The Effects of Sex Hormone Treatment on Ocular Surface Symptoms, Sensitivity and Clinical Signs in Postmenopausal Women with Dry Eye

5.1 Introduction

Several studies have evaluated hormone replacement therapy (HRT), predominantly examining the efficacy of oestrogen, or oestrogen and progesterone, as treatments for dry eye in postmenopausal women. These studies have shown contradictory results (Table 1.2). The randomised placebo-controlled double-masked studies on postmenopausal women with dry eye that showed improvement in signs or symptoms include a daily application of topical oestradiol ointment (dosage not stated) (Akramian et al 1998); a drop to the eye of 9 µg of oestradiol every six hours for four weeks (Sator et al 1998); and a daily phytoestrogen (which affects androgenic activity as described in 1.3.3) tablet for 30 days (Scuderi et al 2012). However, no improvement in dry eye was recorded with a daily combination of 50 mg oestradiol and 2.5 mg medroxyprogesterone acetate for three months in another placebo randomised controlled study which according to the investigators, might be due to the limited number of subjects (Piwkumsribonruang et al 2010). Of note, the comparison of serum or plasma level before and after treatment was not performed in these studies.

The serum concentrations of oestradiol, androgen and the ratio of oestradiol to androgens were not the consistent predictors of symptoms in the untreated post postmenopausal women with dry eye as discussed in Chapter 4. However, a higher level of oestrogen was positively associated with greater corneal staining. In contrast, topical or systemic testosterone therapy has been demonstrated by other non placebo randomised controlled investigations to result in an improvement in dry eye symptoms and signs. For instance increased tear volume was demonstrated in subjects with autoimmune diseases, including Sjögren's Syndrome (Bizzarro et al 1987), after 60 days of oral testosterone. In addition, less dry eye symptoms were reported (with

greatest relief in postmenopausal women) in symptomatic subjects of both genders (age range 25 – 76 years) with 3% testosterone transdermal application around the eye twice daily for two weeks (Connor 2003). Furthermore, an improvement in tear function was proven with a 3-week testosterone patch therapy in women with evaporative dry eye and low testosterone level (Nanavaty et al 2013). Apart from testosterone alone, an improvement in dry eye symptoms with a combination of testosterone and oestrogen in postmenopausal women (Scott et al 2005) was reported in a retrospective study. Administration of dose dependent androgens has inhibited oestrogen from binding to its receptor (Jordan et al 1977) on meibomian gland and therefore reduces the negative effect of oestrogen on the gland. These findings support the relevance of testosterone in dry eye treatment. However, there is no report on dry eye improvement with testosterone supplement in a double-masked randomised placebo-controlled intervention study in a dry eye population.

Psychosocial factors might also confound the relationship between hormone levels and symptoms of dry eye as described in Section 4.1. The Menopause-Specific Quality of Life (MENQOL) questionnaire is able to elicit the effect of the psychological and social factors which includes the domains of vasomotor, psychosocial, physical and sexual symptoms (Hilditch et al 1996) on dry eye. Relationships have been shown between the psychosocial (Mocci et al 2001),sexual domains (Stadberg et al 2000); and systemic symptoms (Shimmura et al 1999) with dry eye. Therefore, the effects of sex hormone intervention on systemic symptoms and quality of life based on MENQOL domain scores were investigated in this chapter.

To better confirm the absorption of the hormone into the circulation in an intervention study, the sex hormone concentration should be measured prior to and immediately after the treatment duration. ADT-G and 3α-diol G are proposed as appropriate markers of the androgenic activity in postmenopausal women (Labrie et al 1998) while DHEA-S is an important androgen precursor in the intracrinology process (Labrie 2010) as described in Sections 1.4.1 and 4.1. Therefore, it would be appropriate to monitor the physiological changes to such androgen metabolites and precursor in an intervention study on hormone treatment in this population. This chapter presents a pilot investigation of the effects of transdermal androgen and/or oestrogen treatment on postmenopausal women with dry eye using a randomised placebo controlled

interventional trial design. Clinicians should be more cautious with these potential effects on the clinical signs measured especially in the hormone treatment receivers. Non inclusion of progesterone hormone in this interventional study was due to the lack of significant associations between the hormone and the variables in the earlier studies in the thesis.

5.2 Aim

To determine which treatment among testosterone, oestrogen and their combination shows the greatest effect (improve or worsen) on ocular surface symptoms; systemic symptoms and quality of life; ocular surface sensitivity and clinical signs of dry eye in a randomised placebo-controlled double-masked study of eight weeks duration.

5.3 Hypotheses

i. Those receiving oestradiol show worse ocular surface symptoms; MENQOL domain scores; reduced ocular surface sensitivity and worse clinical signs of dry eye post therapy.

ii. Those receiving testosterone and the combined testosterone and oestrogen show, improved ocular surface symptoms; MENQOL domain scores; increased ocular surface sensitivity and improved clinical signs of dry eye post therapy.

5.4 Method

5.4.1 Study Design

An exploratory randomised-placebo-controlled double-masked study involving 40 subjects who attended two visits (baseline and final) eight weeks apart was designed with the subjects visit tracking record displayed below. Ten subjects were randomly assigned to one of four groups and received the following treatment daily:

Group 1 Testosterone Cream and placebo gel
Group 2 Oestradiol gel and placebo cream
Group 3 Testosterone cream and oestradiol gel
Group 4 Placebo cream and gel

SUBJECTS VISIT TRACKING RECORD

First Visit	Subject Discontinuation	Subject Replacement	Final Visit
Group 1 (Testosterone) DE01 DE02 DE10 DE14 DE17 DE20 DE22 DE28 DE36 DE40	No discontinuation	None	DE01 DE02 DE10 DE14 DE17 DE20 DE22 DE28 DE36 DE40 n=10
Group2 (Oestradiol) DE03 DE04 DE11 DE12 DE19 DE24 DE26 DE31 DE32 DE34	DE03: Complained of breast tenderness after 3 weeks of application. Stopped application at week 4. Problem resolved within 2 weeks after termination of supplement. DE26: Prolonged influenza. Stopped application at week 5. Continued having influenza for a week after termination of supplement. DE32:Constipation.Stopped application 3 days after baseline visit. Problem resolved within 6 weeks after termination of supplement. DE34: Subject decided to withdraw after the baseline visit. Did not start with the supplement.	DE42 DE43 DE44 DE45	DE42 DE04 DE11 DE12 DE19 DE24 DE43DE31 DE44 DE45 n=10

Group 3 (Combination of testosterone and oestradiol) DE08 DE09 DE13 DE21 DE23 DE25 DE27 DE30 DE39 DE37	DE37: Subject decided to withdraw due to a family problem. Stopped application at week 4.	DE 46	DE08 DE09 DE13 DE21 DE23 DE25 DE27 DE30 DE39 DE46 n=10
Group 4 Placebo DE05 DE06 DE07 DE15 DE16DE18 DE29 DE33 DE35 DE3	E06: Subject decided to withdraw after the baseline visit. Did not start with the supplement.	DE 41	DE05 DE41 DE07 DE15 DE16 DE18 DE29 DE33 DE35 DE38 n=10

5.4.2 **Treatment**

The testosterone treatment consisted of AndroFeme® 1% testosterone (Lawley Pharmaceuticals Pty Ltd Australia). A daily dosage of 0.5 mL of white cream was dispensed from a tube using an extractor (Figure 5.1) and applied daily. Sandrena (Aspen Pharmacare Australia) is a transparent gel 0.1% (w/w) (percentage weight/weight) with 0.1 gm of oestradiol solute in 100 gm of solution, which was distributed in individual syringes for the purpose of this study so as to ensure masking, containing the daily dosage of 1 mg. The placebo gel and cream were dispensed in syringes (Figure 5.2) and tubes similar to Sandrena and AndroFeme, respectively, for double masking purposes.

Figure 5.1 Extractor used for the Androfeme and placebo cream (Lawley Pharmaceuticals Pty Ltd Australia)

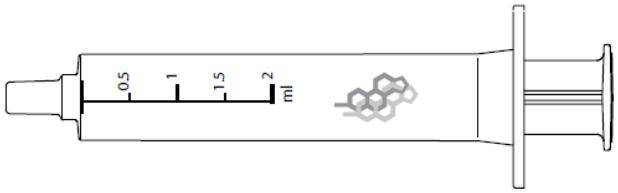
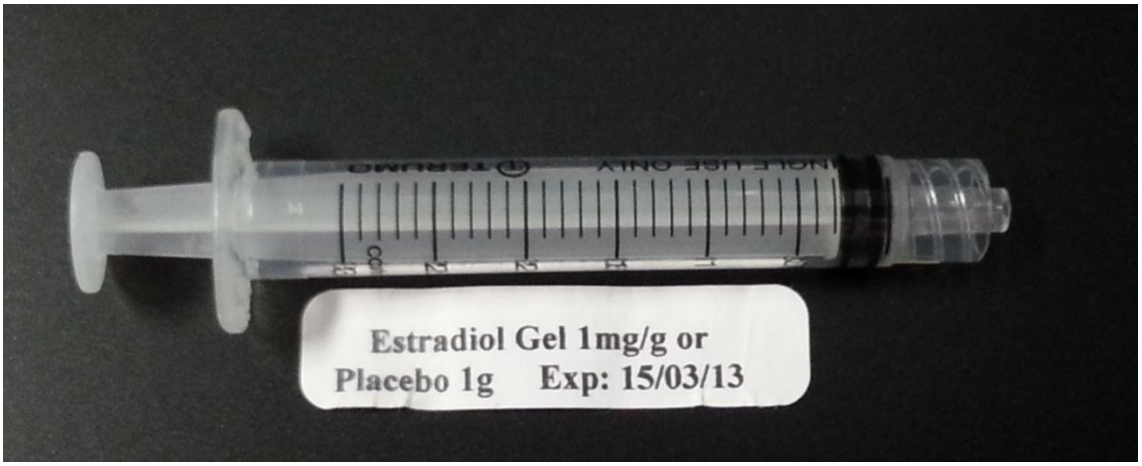


Figure 5.2 Syringe containing oestrogen or placebo gel



5.4.2.1 Randomisation of Treatment

Forty tickets numbered 1 to 4 (representing treatment groups) were randomly allocated to and later sealed in envelopes numbered 1 to 40 (representing the 40 subjects) while the final six subjects were replacements using numbered tickets 41 to 46. This task was completed by an unmasked investigator who was uninvolved in data collection. At the end of the baseline visit, the treatments were dispensed to subjects by a masked investigator. The investigators undertaking the data collection and analysis were masked to the group allocation until all data analysis had been completed.

5.4.2.2 Application of Treatment

The daily treatments were applied onto subjects' inner thighs. Cream was applied to one thigh and gel was applied to the other (figure 5.2). Instructions were provided in the patient consent form (Appendix 3).

5.4.3 Subjects

This study consisted of forty subjects who completed the study described in Chapter 4. Ethics approval and subject enrolment and recruitment were described in Section 4.4.1. Signed informed consent was obtained from each subject prior to enrolment in the study.

Inclusion criteria

- Female gender
- Age 50 years and above
- Permanent menstrual cessation for at least 1 year
- Diagnosis of dry eye based on Women's Health Study criteria (Schaumberg et al 2001)

Exclusion criteria

- Sjögren's syndrome based on European Classification of Sjögren's syndrome (Vitali et al 2002) (Appendix D) based on all of the following criteria
- Sjögren's syndrome based on European Classification of Sjögren's syndrome (Vitali et al 2002) (Appendix D) based on all of the following criteria
  i. shows positive response to at least one of the ocular symptoms questions,
  a) Have you had daily, persistent, troublesome dry eyes for more than 3 months?
  b) Do you have a recurrent sensation of sand or gravel in the eyes?
  c) Do you use tear substitutes more than 3 times a day?
  ii. shows positive response to at least one of the oral symptoms questions
  a) Have you had a daily feeling of dry mouth for more than 3 months?
  b) Have you had recurrently or persistently swollen salivary glands as an adult?
  c) Do you frequently drink liquids to aid in swallowing dry food?
  iii. less than 5 mm of wetted Schirmer test strip in 5 minutes

  Subjects who show positive responses to i and ii and iii are considered as potential Sjögren's syndrome patient and is confirmed with

  iv. the presence of antibodies to Ro(SSA) or La(SSB) antigens, or both in the serum
- History of corneal or refractive surgery
- History of hormone therapy within the past 12 months
- History of hysterectomy and/or oopherectomy
- Prior diagnosis of infectious disease transmittable by blood (eg HIV/AIDS, Hepatitis)
- Eye surgery within the past 6 months immediately prior to enrolment for this study
- Use of antidepressant medication if first started within the past 12 months
- Subjects with ocular or systemic disease and/or associated treatment deemed likely to significantly impact on the ocular surface
- Use of systemic or topical medication likely to significantly affect ocular physiology (eg anti-acne medications such as Roaccutane, corticosteroid or

immunosuppressant medications such as Hydrocortisone, Prednisolone, and antihistamine medications)

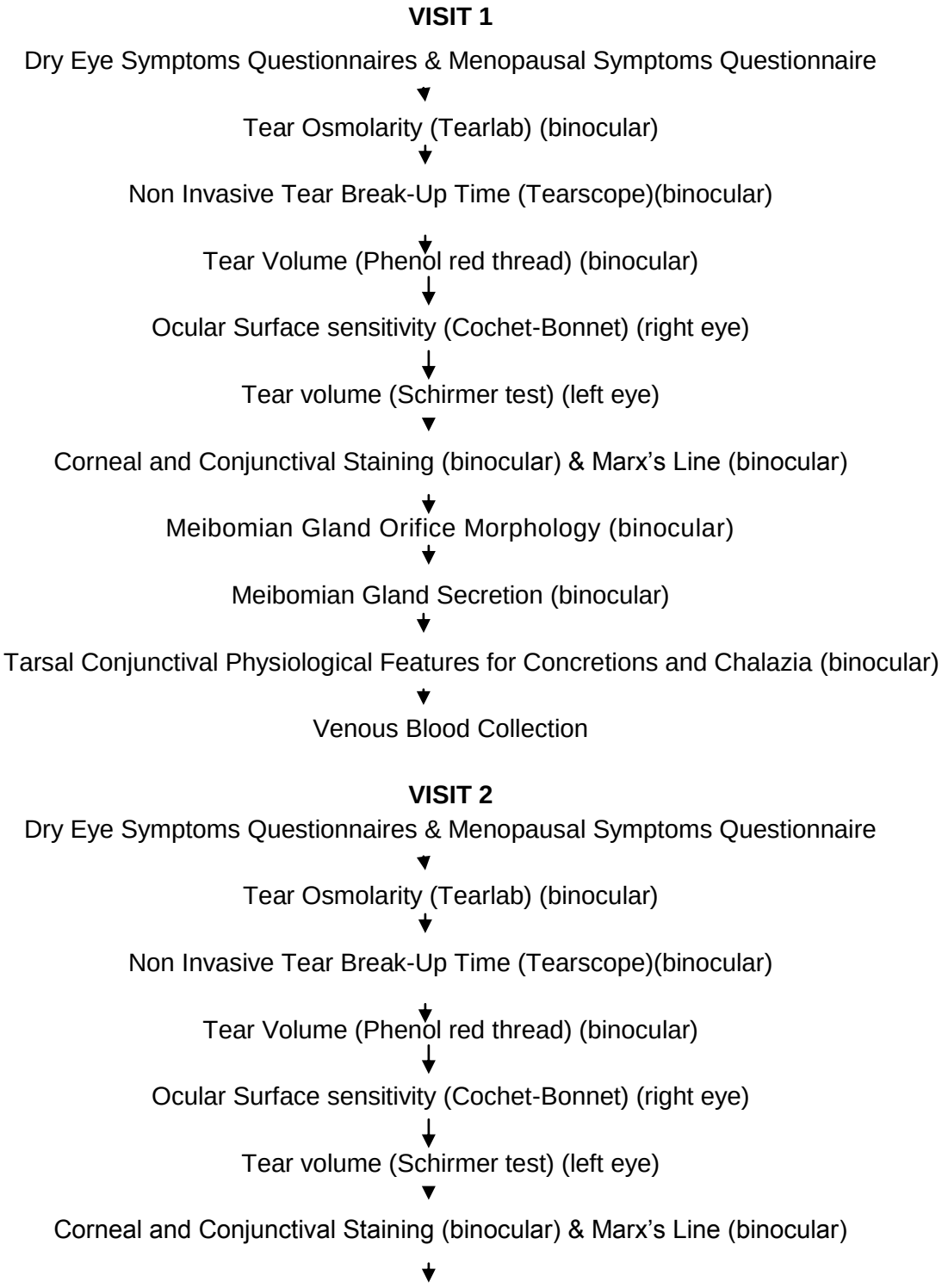
- Use of systemic or topical medication whose effect may be reduced by oestrogen therapy (eg anticonvulsants, meprobamate, phenylbutazone, griseofulvin and rifampicin)
- Subjects with systemic disease and/or associated treatment which is likely to be significantly affected by the treatment product for example:
    - Severe hepatic and renal disease
    - Known or suspected carcinoma of the breast, endometrium or other oestrogen dependent neoplasia
    - High blood pressure
- Any changes to ocular or systemic medication or regimen during the course of the study.
- Known hypersensitivity to oestrogens, testosterones or any other component of the Sandrena gel or AndroFeme cream.
- Any changes to ocular or systemic medication or regimen during the course of the study.
- Known hypersensitivity to oestrogens, testosterones or any other component of the Sandrena gel or AndroFeme cream.

Subjects were required to inform the investigators of any changes to ocular or systemic medication or regimen during the course of the study.

5.4.4 Measurements of Sex Hormone Levels, Ocular Symptoms, Sensitivity and Clinical Signs

The measurements of sex hormone concentrations; ocular surface symptoms; MENQOL domain scores; ocular surface sensitivity and clinical signs performed in this study are described in Section 4.4.3. The measurements of study variables and venous blood collection were carried out at baseline and eight weeks post treatment. Both study visits consisting of tests listed below, were scheduled at the same time of day to mitigate the effects of diurnal variation.

5.4.4.1 Flow chart of tests performed on subjects on both visits



Meibomian Gland Orifice Morphology (binocular)

↓

Meibomian Gland Secretion (binocular)

↓

Tarsal Conjunctival Physiological Features for Concretions and Chalazia (binocular)

↓

Venous Blood Collection

5.5 Statistical Analysis

Normality of data was assessed with Kolmogorov-Smirnov test (p>0.05). Differences between groups at baseline were assessed with One-way ANOVA or Kruskal-Wallis tests (p<0.05).The establishment of the impact of intervention was based on the differences of the scores (final visit minus base line) and assessed with One-way ANOVA which was followed by Dunnett t test (p<0.1) to establish significant differences between the oestradiol, testosterone and combined treatment groups and placebo (p<0.1). Comparison of study variables between visits (change with time) were assessed by paired t-test or Wilcoxon-signed rank test (p<0.1). A less stringent p value (p<0.1) was selected based on the relatively small sample size, statistical power considerations and exploratory purpose of the study (Henderson et al 2000).

5.6 Results

Forty subjects completed the study. The age range of the moderate dry eye subjects was 53 to 83 years (mean 64.2 ± 5.3 years). None of the subjects were contact lens wearers. One subject had an ovary removed by surgery.

There were four dropouts from the oestrogen treatment group as follows: One subject discontinued after 3 weeks of treatment due to breast tenderness. The condition resolved within 2 weeks after discontinuation.

One subject discontinued after 5 weeks of treatment due to prolonged influenza and continued having influenza for a week after discontinuation.

One subject discontinued after 3 days of treatment due to constipation. The condition resolved within 6 weeks after discontinuation.

One subject discontinued from the study after the baseline visit, prior to dispensing of treatment.

There were no discontinuations from the testosterone treatment group. One subject decided to withdraw due to a family problem after 4 weeks of treatment in the combined treatment group. One subject in the placebo group was concerned about the potential side effects of treatment had decided to with draw after the baseline visit.

The six subjects who discontinued from the study were replaced and the replacement subjects allocated to the same group.

5.6.1 Normality of Study Variables at Baseline

Table 5.1 shows the Kolmogorov-Smirnov test for normality of distribution. Variables with $p \geq 0.05$ have a normal distribution. Normally distributed variables are indicated in bold.

Table 5.1 Normality of Study Variables (n=40)

Variables	Kolmogorov-Smirnov (p values)
Sex Hormone Levels	
Oestradiol (pg/mL)	< 0.001
3α-diol G (ng/ml)	< 0.001
DHEA-S (µg/ml)	< 0.001
Symptoms	
OSDI	**0.05**
OCI	**0.20**
OCI Dryness Intensity	< 0.001
OCI Dryness Frequency	< 0.001
MENQOL domain scores	
Psychosocial	< 0.001
Physical	< 0.001
Vasomotor	< 0.001
Sexual	**0.15**
Ocular Surface Sensitivity	
Central cornea [(CCS ($1/g/mm^2$)] (Right Eye)	< 0.001
Inferior conjunctiva [(ICJS ($1/g/mm^2$)] (Right Eye)	< 0.001
Clinical Signs	
Tear osmolarity [(mOsms/L) Worst Eye]	< 0.001
Tear Volume [PRT (mm)(Average of both eyes)]	**0.12**
Tear Volume [(Schirmer (mm)(Left Eye)]	< 0.005 (0.004)
Non invasive tear break-up time [NIBUT(s)(Average of both eyes)]	0.02
Corneal Staining [(grade) (Average of both eyes)]	< 0.001
Conjunctival Staining [(grade)(Average of both eyes)]	**0.11**

Marx's Line [(grade)(Average of both eyes)]	< 0.001
Vascularity of lower lid margin [(grade)(Average of both eyes)]	< 0.001
Telangiectasia on lower lid margin [(grade)(Average of both eyes)]	< 0.001
Meibomian gland Expressibility on lower lid margin [(grade)(Average of both eyes)]	< 0.001
Number of glands on lower lid margins [(number)(Average of both eyes)]	< 0.001
Number of Capped glands on lower and upper lid margins[(number)(Average of both eyes)]	.< 0.001

5.6.2 **Baseline Study Variables**

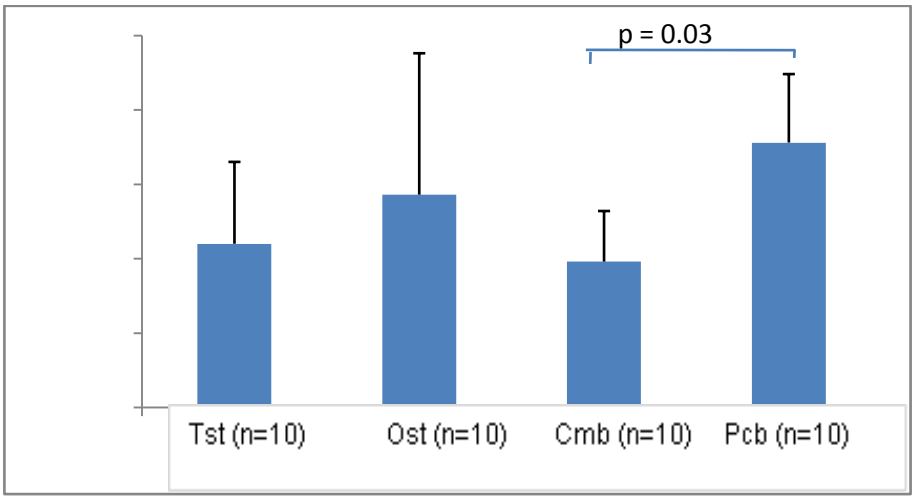
Table 5.2 displays the means and standard deviation or the range and median of the study variables, age and the duration of menopause. There were differences between groups in corneal staining and the number of years since menopause. In all tables and figures presented below, group 1 represents testosterone treatment, group 2 represents oestrogen treatment, group 3 represents the testosterone and oestrogen combined treatment and group 4 represents the placebo treatment.

Table 5.2 Study variables at baseline. Significant p values are indicated in bold. Asterisk represents variables with significant differences between groups

Variables	Group 1	Group 2	Group 3	Group 4	p values
	Means ± SD(Ranges) IQR/Median				
Age (years)	63.5 ± 4.5	65.2 ± 7.5	61.6 ± 4.0	66.4 ± 3.8	0.21
Years since Menopause	11.0 ±5.5	14.3± 9.5	*9.8 ±3.4	*17.8± 4.6	**0.03**
Sex Hormone Levels					
Oestradiol(pg/mL)	(19.9±36)/ (2.5-108.1) 6.4/12.0	(14.0±16.9) (2.8-49.6)/5.2/16.8	(4.1±1.9) (2.3-7.3)/3.6/3.3	(10.0±9.3) (1.8-32.6)/6.6/8.2	0.30
3α-diol G(ng/mL)	(2.6±4.2) (0.4-14.4)/1.3/1.6	(2.2±1.5) (1.0-5.9)/1.6/1.1	(1.3±0.7) (0.6-2.8)/1.0/0.9	(3.0±3.8) (1.0-13.4)/1.8/1.7	0.20
DHEA-S(µg/mL)	(1.0±0.7) (0.1-2.5)/0.7/1.0	(0.7±0.3) (0.4-1.5)/0.5/0.3	(0.5±0.2) (0.3-0.9)/0.5/0.3	(0.9±0.8) (0.3-2.9)/0.7/0.5	0.40
Symptoms					
OSDI	32.3 ± 19.2	26.8 ± 23.7	24.5 ± 12.5	30.7 ±18.4	0.87
OCI	42.6 ± 8.6	35.8 ± 10.1	38.0 ± 10.1	34.0 ±8.7	0.21
OCI dryness Intensity	3.9± 1.4	2.8 ±1.5	3.6± 1.0	4.0± 0.8	0.51
OCI dryness frequency	4.7± 1.1	3.7 ±2.3	4.2± 1.4	4.7± 1.4	0.16
MENQOL domain scores					
Psychosocial	2 ± 1	2 ± 1	2 ± 1	2 ± 1	0.68
Physical	3 ± 1	3 ± 1	3 ± 1	3 ± 1	0.43
Sexual	2 ± 2	2 ± 1	2 ± 2	1 ± 1	0.46
Vasomotor	2 ± 1	3 ± 2	3 ± 2	3 ± 2	0.28
Ocular Surface Sensitivity					
Central cornea [(CCS (1/g/mm^2)]	3.2± 3.5	2.9 ±3.2	3.1± 3.2	3.8± 3.7	0.99
Inferior conjunctiva [(ICJS (1/g/mm^2)]	0.6±1.1	0.4±0.5	1.2± 2.8	0.6±1.1	0.5
Clinical Signs					
Tear osmolarity(mOsms/L)	302.1± 5.4	307.2± 16.6	310.0 ±18.3	310.1± 10.9	0.56

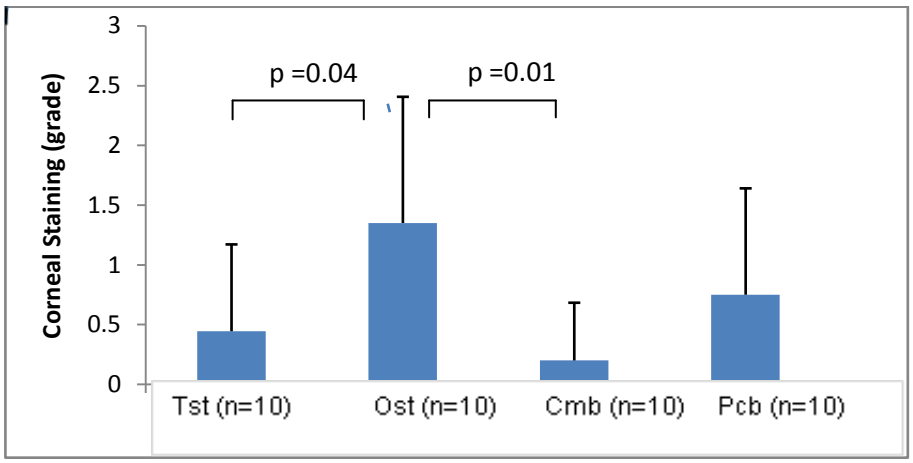
Variables	Group 1	Group 2	Group 3	Group 4	p values
Tear Volume [PRT (mm)]	23.1 ±4.9	23.5± 6.2	24.8 ±3.8	26.6± 3.4	0.27
Tear Volume [(Schirmer (mm)]	9.3± 4.3	14.0± 11.4	12.6 ±9.4	12.2± 7.3	0.97
Non invasive tear break-up time [NIBUT(s)]	9.4 ±2.8	10.5± 5.4	10.0± 2.1	9.5± 2.5	0.63
Corneal Staining (grade)	*(0-1)/0/0.5	*(0-3)/1.5/2	(0-1.5)/0/0.4	(0-2)/1.5/1.3	**0.03**
Conjunctival Staining (grade)	(1-7)/1.5/2.8	(0-5)/2.5/2	(0-6)/1.8/4.6	(0-6)/0/2.3	0.92
Marx's Line (grade)	0.4±0.7	0.8±1.5	1.6 ±2.3	1.5 ± 1.9	0.23
Vascularity of lower lid margin (grade)	(0-1.8)/1.0/1.4	(0-2)/1.0/1.3	(0-1.5)/0/0.5	(0-1.8)/0.5/1.6	0.66
Telangiectasia on lower lid margin (grade)	(0-1.5)/0/1.0	(0-2)/0/0.8	(0-0.5)/0/0	(0-1)/0/0.6	0.66
Meibomian gland expressibility on lower lid margin (grade)	(0-4)/0/3	(0-6)/0/4.5	(0-3.5)/0/2.3	(0-6)/0/4	0.89
Number of glands on lower lid margins (number)	(0-16)/5/3.5	(0-13)/5/2	(0-4)/5/2	(0-4)/5/2.5	0.65
Number of capped glands on lower and upper lid margins (number)	(2-6)/0/9.5	(2-6)/0/2.5	(3-8)/0/1	(5-8)/1/1	0.65

Figure 5.3 Years since menopause in all groups (n=40)



Years since menopause were significantly greater in group 4 than group 3 (p=0.03)

Figure 5.4 Corneal staining score at baseline (n=40).



Corneal staining was significantly higher in group 2 than in group 1(p= 0.04) or 3 (p= 0.01)

5.6.3 **Effect of Intervention**

Differences in the scores (final – baseline) of the study variables within the four groups were initially compared based on their normality (Tables 5.3 and 5.4). Significant differences were identified in the oestradiol concentration (p<0.001), the ratio of oestradiol to 3α-diol G concentration (p<0.001), OCI dryness intensity (p=0.07) and corneal staining (p=0.01). Individual comparison of these variables between each treatment group and placebo was further investigated and the results are plotted in Figures 5.5, 5.8, 5.13 and 5.18 respectively.

Table 5.3 Changes in the study variables (parametric) (n=40)

Variables	Between Groups df	F	P value between groups
Ocular Comfort Index	3	0.89	0.47
Ocular Surface Disease Index	3	0.81	0.51
Tear osmolarity[(mOsms/L) Worst Eye]	3	1.29	0.29
Tear Volume [Phenol Red Thread (mm) (Average of both eyes)]	3	1.81	0.16
Tear Volume[(Schirmer (mm) (Left Eye)]	3	0.70	0.56
Non invasive tear break-up time [NIBUT(s)(Average of both eyes)]	3	0.33	0.80
Conjunctival Staining [(grade)(Average of both eyes)]	3	0.39	0.76
Sexual domain	3	2.22	0.10

Table 5.4 Changes in the study variables (non-parametric) (n=40)

Variables	Chi Square	df	p values between groups
Ocular Comfort Index dryness frequency	1.11	3	0.77
Ocular Comfort Index dryness intensity	6.99	3	**0.07**
Oestradiol (pg/mL)	23.84	3	**<0.001**
Oestradiol : 5alpha-androstane-3alpha and 17beta-diolglucuronide (3α-diol G)	19.07	3	**<0.001**
5alpha-androstane-3alpha and 17beta-diolglucuronide (3α-diol G) (ng/mL)	4.03	3	0.26
Dehydroepiandrosterone Sulfate (DHEA-S) (µg/mL)	0.32	3	0.96
Psychosocial	1.65	3	0.65
Physical	1.82	3	0.61
Vasomotor	1.23	3	0.75
Central cornea sensitivity [CCS(1/g/mm^2)](Right Eye)	0.57	3	0.90
Inferior conjunctiva sensitivity [ICJS(1/g/mm^2)] (Right Eye)	0.73	3	0.87
Corneal Staining [(grade) (Average of both eyes)]	11.72	3	**0.01**
Marx's Line [(grade)(Average of both eyes)]	0.45	3	0.93
Vascularity of lower lid margin [(grade)(Average of both eyes)]	5.41	3	0.14
Telangiectasia on lower lid margin [(grade)(Average of both eyes)]	1.80	3	0.61
Meibomian gland Expressibility on lower lid margin [(grade)(Average of both eyes)]	3.74	3	0.29
Number of glands on lower lid margins [(number)(Average of both eyes)]	5.336	3	0.15
Number of Capped glands on lower and upper lid margins [(number)(Average of both eyes)]	1.681	3	0.64

5.6.3.1 Sex Hormone Concentrations

The comparisons of the changes in the serum sex hormone concentrations, ratio of oestradiol to androgen between each treatment group and placebo are shown in Figures 5.5 and 5.8. The change in serum oestradiol concentration was significantly greater in groups 2 (p=0.03) and 3 (p=0.01) than in placebo and the ratio of oestradiol to androgen was also significantly greater in groups 2 (p=0.02) and 3 (p=0.01) than in placebo. There were no significant differences between treatment groups for changes in DHEA-S and 3α-diol G serum concentrations (Figures 5.6 and 5.7).

Figure 5.5 Changes in oestradiol concentration for each treatment group

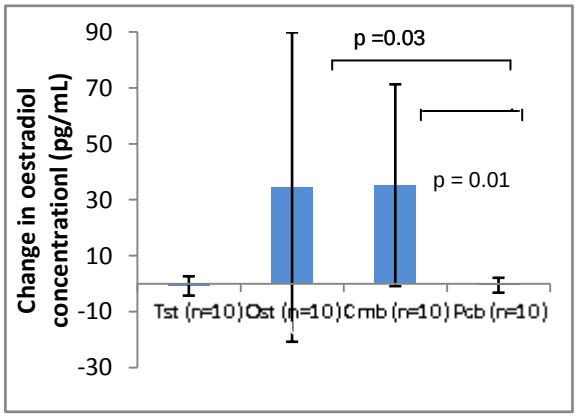


Figure 5.6 Changes in 3α-diolG concentration for each treatment group

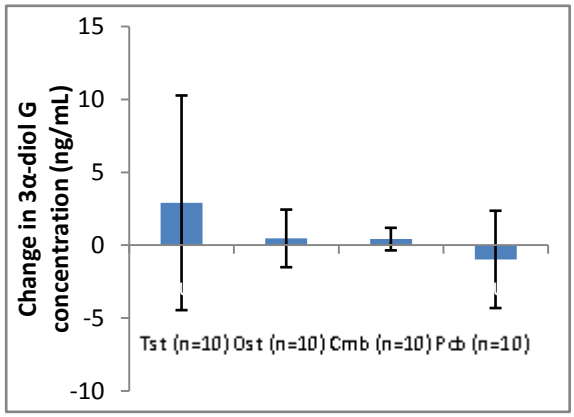


Figure 5.7 Changes in DHEA-S concentration for each treatment group

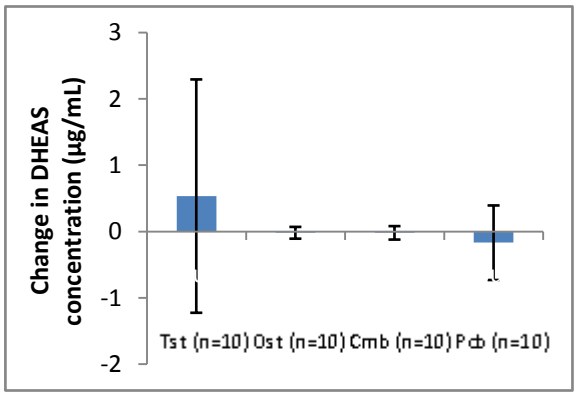
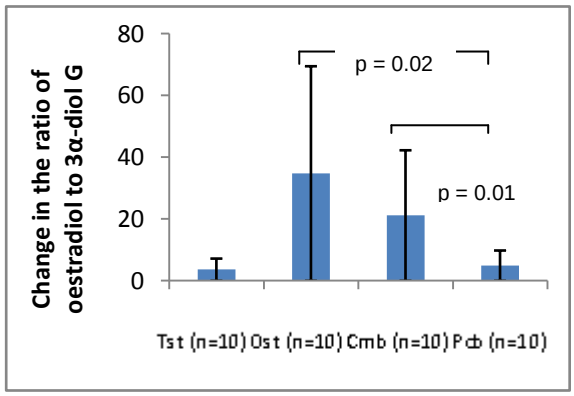


Figure 5.8 Changes in the ratio of oestradiol to 3α-diol G for each treatment group



5.6.3.2 Ocular Symptoms

The comparisons of the changes in the ocular symptoms between each treatment group and placebo are shown in Figures 5.12. The only difference in symptoms with intervention was the less improvement in OCI dryness intensity with oestrogen than with placebo (p=0.06). The change in OCI dryness intensity in the testosterone and combined testosterone/oestrogen groups was not significantly different to placebo (Figure 5.13). Changes in the other ocular symptoms scores were not significantly different between groups (Figures 5.9 to 5.11).

Figure 5.9 Change in OSDI for each treatment group

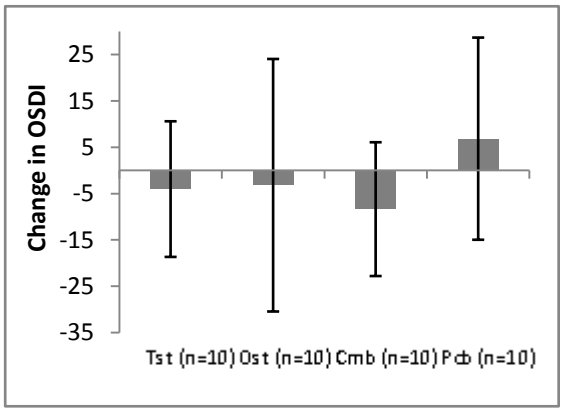


Figure 5.10 Change in OCI for each treatment group

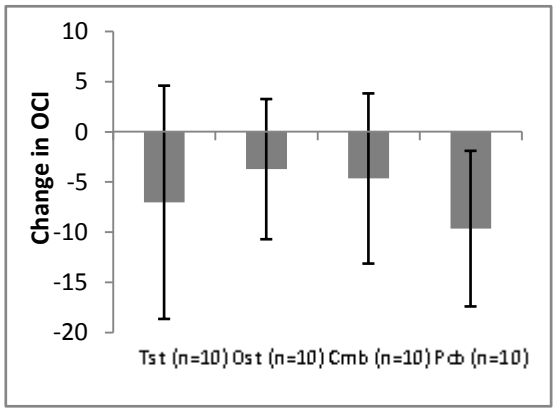


Figure 5.11 Change in OCI frequency of dryness for each treatment group

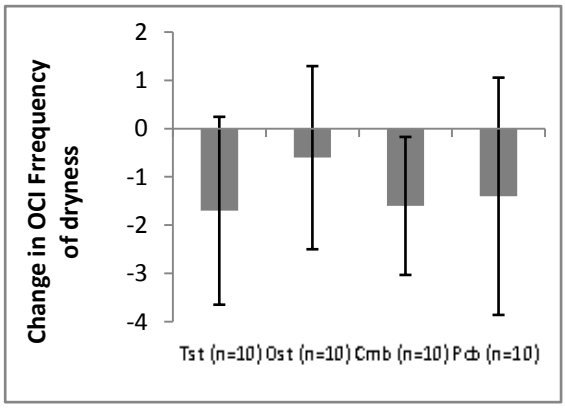
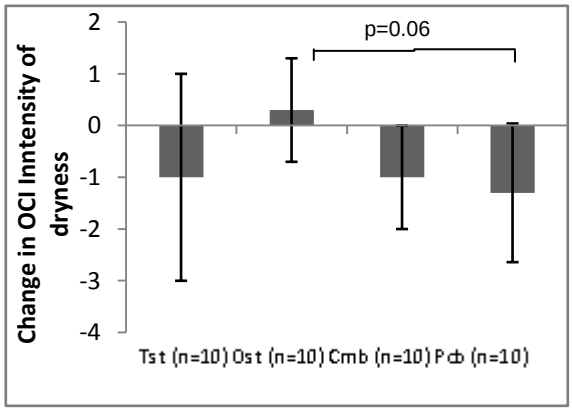


Figure 5.12 Change in OCI intensity of dryness for each treatment group



5.6.3.3 MENQOL domain scores

Changes in MENQOL scores were not significantly different between groups. The comparisons of changes in the MENOQL domain scores between treatment groups are shown in Figures 5.13 to 5.16.

Figure 5.13 Change in psychological domain score for each treatment group

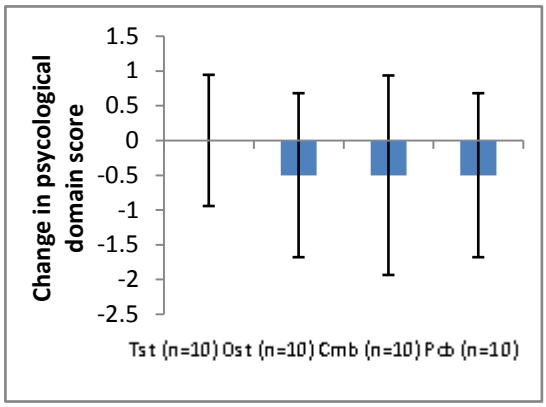


Figure 5.14 Change in physiological domain score for each treatment group

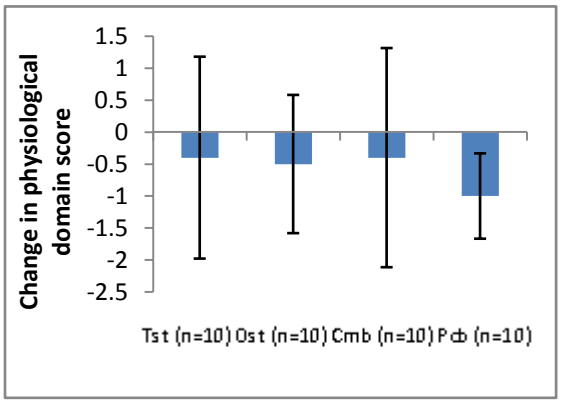


Figure 5.15 Change in vasomotor domain score for each treatment group

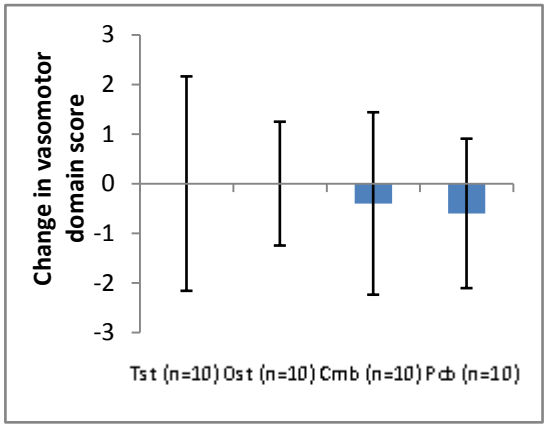
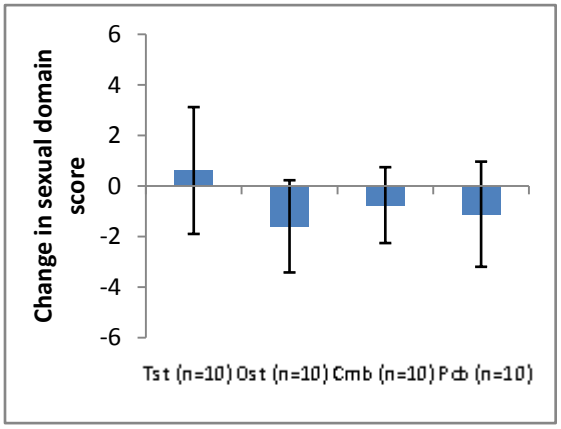


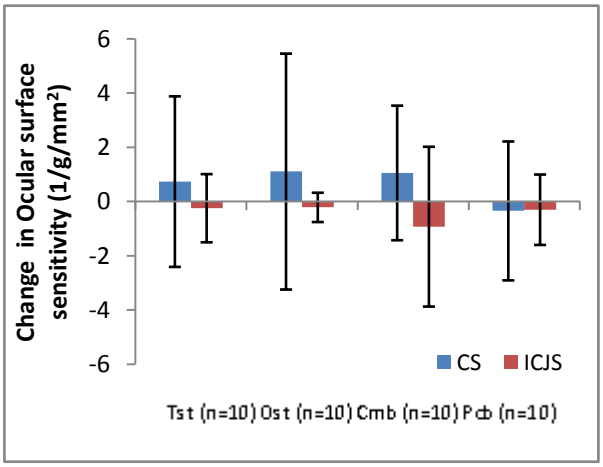
Figure 5.16 Change in sexual domain score for each treatment group



5.6.3.4 Ocular Surface Sensitivity

The comparisons on the changes in the ocular surface sensitivity between treatment groups are shown in Figure 5.17. These changes were not significantly different between groups.

Figure 5.17 Changes in corneal sensitivity (CS) and inferior conjunctival sensitivity (ICJS) for each treatment group



5.6.3.5 Clinical Signs

The comparisons of the changes in corneal staining between each treatment group and placebo are shown in Figures 5.18.Corneal staining was significantly increased with both testosterone (p=0.01) and the combined treatments (p=0.07), than with placebo whereas the effect of oestrogen was not significantly different to placebo. The changes in conjunctival staining, tear osmolarity, NIBUT and tear volume were not significantly different between groups (Figures 5.19 to 5.22).

Figure 5.18 Changes in the corneal staining for each treatment group

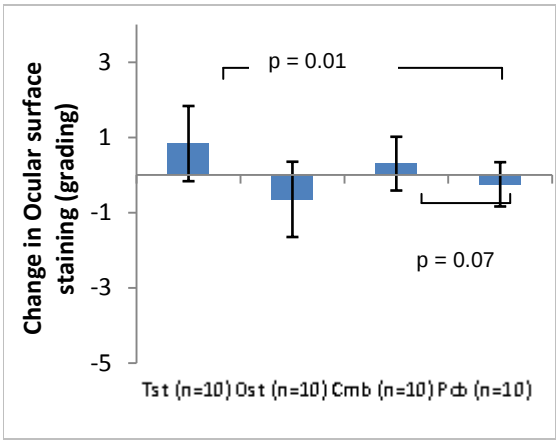


Figure 5.19 Changes in the conjunctival staining for each treatment group

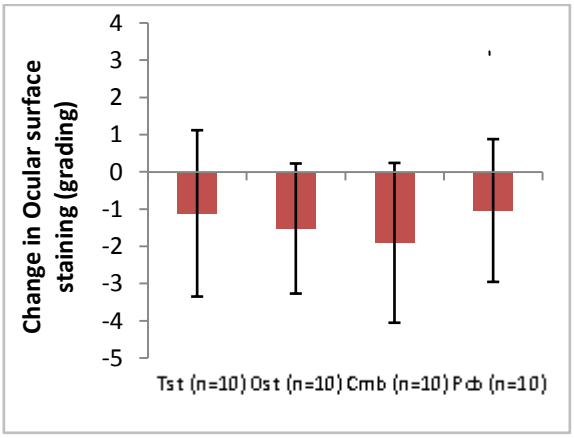


Figure 5.20 Changes in NIBUT for each treatment group

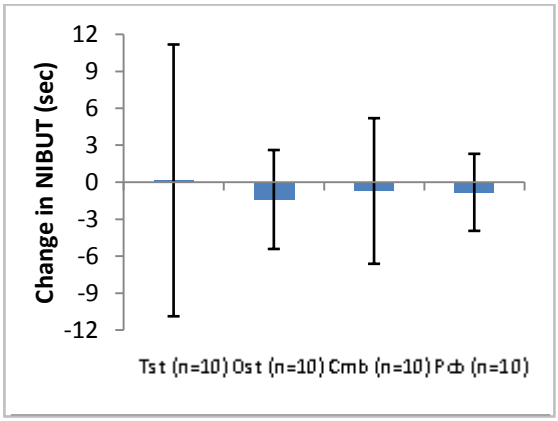


Figure5.21 Changes in tear volume for each treatment group

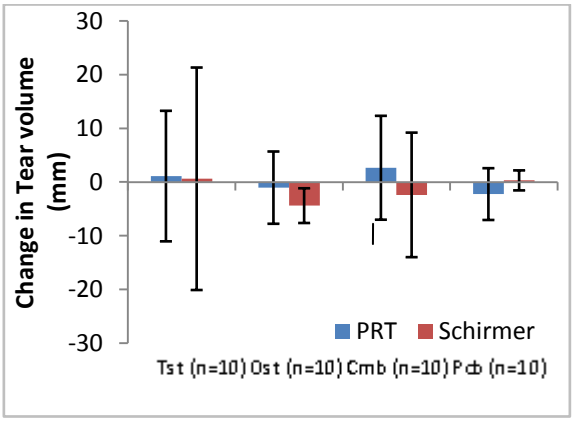
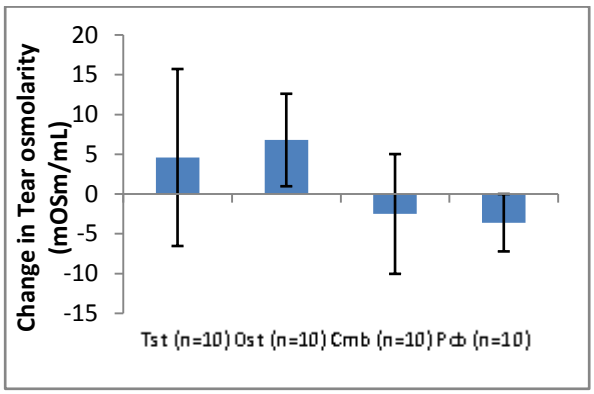


Figure 5.22 Change in tear osmolarity for each treatment group



5.6.4 **Effect of Intervention (Changes over time)**

5.6.4.1 Sex Hormone Concentration

The serum concentration of different sex hormones and ratio of oestradiol to 3α-diol G is shown in Figures 5.23 to 5.26. The concentration of oestradiol was increased in groups 2 (p=0.03) and 3 (p=0.01) post treatment (Figure 5.23). The ratio of oestradiol to 3α-diol G was increased in groups 2 (p=0.02) and 3 (p=0.01) but reduced in group 1 (p=0.01) post treatment (Figure 5.26). However there were no significant changes in the concentrations of DHEA-S and 3α-diol G (Figures 5.24 to 5.26).

Figure 5.23 Mean group oestradiol concentration by visit

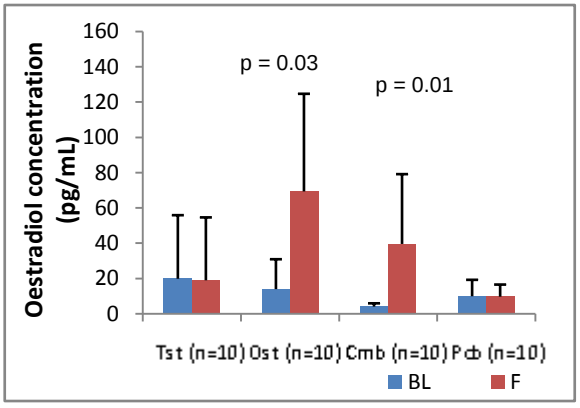


Figure 5.24 Mean group DHEA-S concentration by visit

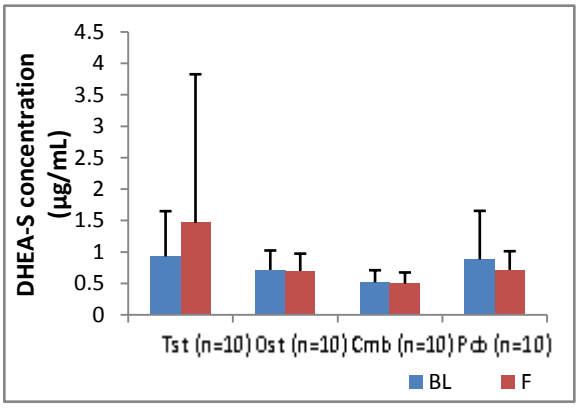


Figure 5.25 Mean group 3α-diol G concentration by visit

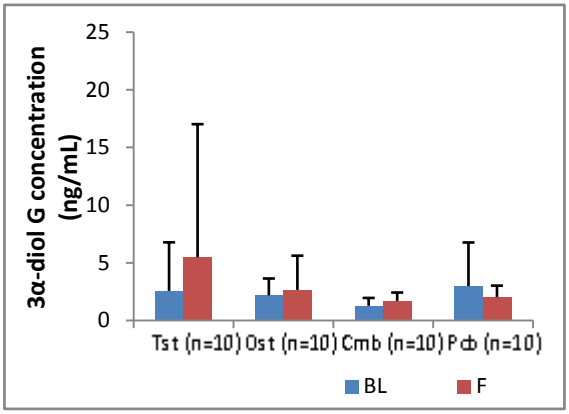
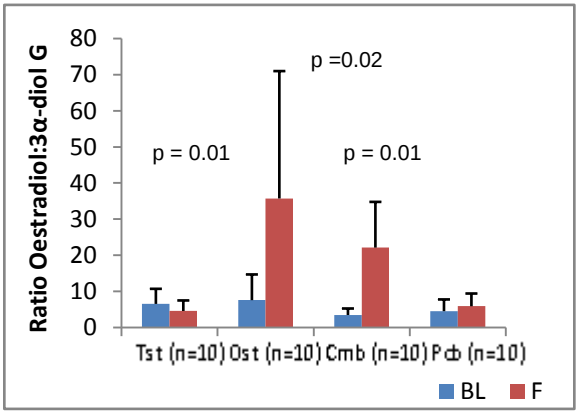


Figure 5.26 Mean group ratio of oestradiol to3α-diol G by visit



5.6.4.2 Ocular Symptoms

All treatment groups showed reduced symptoms (Figures 5.27 to 5.30). OSDI scores reduced in groups 2 (p=0.08) and 3 (p=0.07) (Figure 5.28); OCI dryness frequency scores reduced in groups 1 (p=0.03) and 3 (p=0.01) (Figure 5.29); and OCI dryness intensity scores reduced in groups 3 (p=0.03) and 4 (p=0.02) (Figure 5.30).

Figure 5.27 Mean group OCI scores by visit

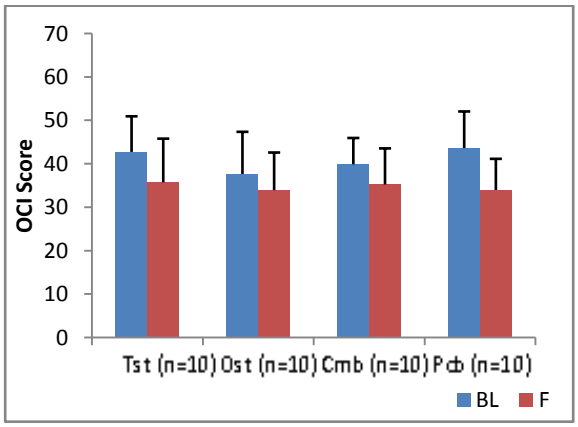


Figure 5.28 Mean group OSDI scores by visit

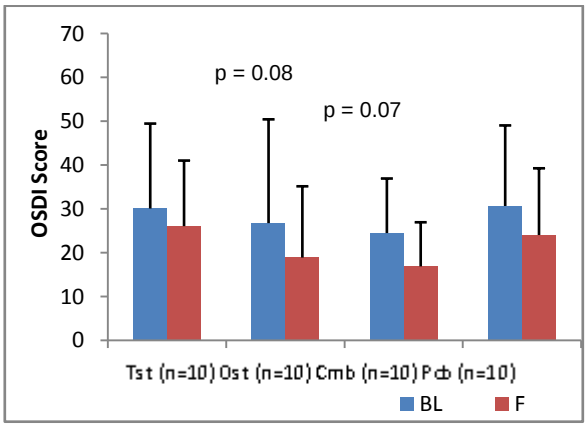


Figure 5.29 Mean group OCI dryness frequency by visit

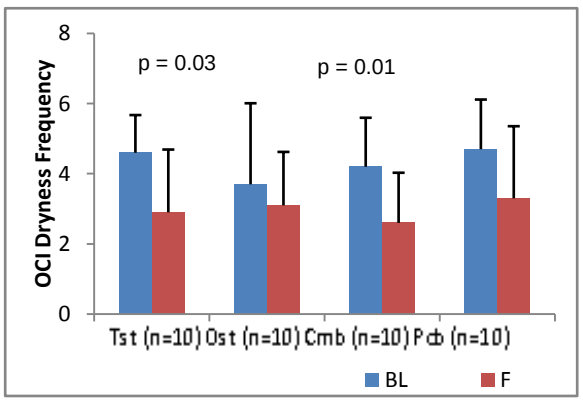
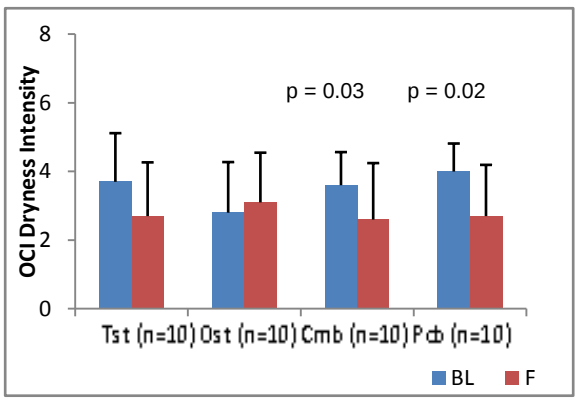


Figure 5.30 Mean group OCI dryness intensity by visit



5.6.4.3 MENQOL Domains Scores

The MENQOL domains scores are shown in Figures 5.31 to 5.34. Sexual domain score was lower (improved) in group 2 (p=0.04) (Figure 5.34) post treatment. However there were no significant differences in the domain scores for other domain scores.

Figure 5.31 Mean group psychosocial domain score by visit

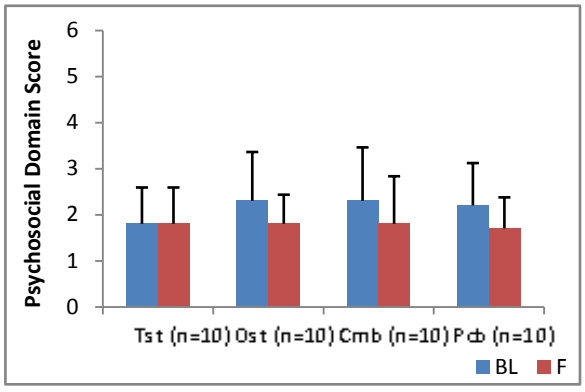


Figure 5.32 Mean group physical domain score by visit

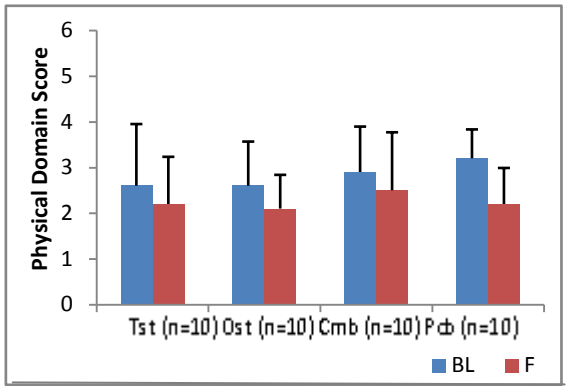


Figure 5.33 Mean group vasomotor domain score by visit

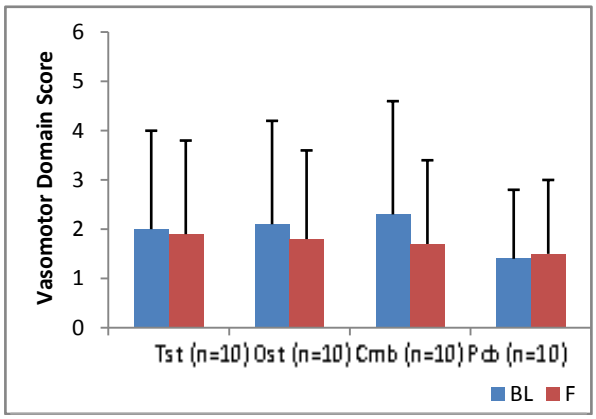
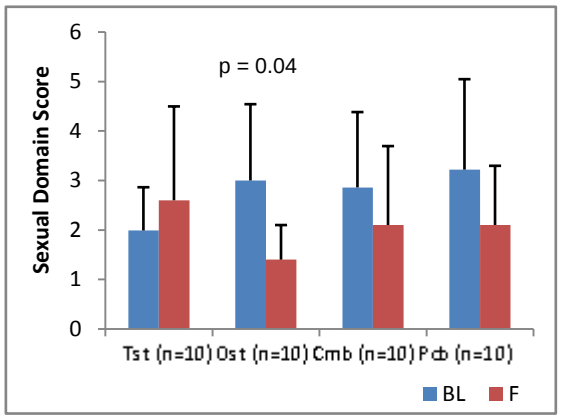


Figure 5.34 Mean group sexual domain score by visit



5.6.4.4 Ocular Surface Sensitivity

There were no changes in corneal or conjunctival sensitivity in any of the treatment groups (Figures 5.35 and5.36)

Figure 5.35 Mean group corneal sensitivity by visit

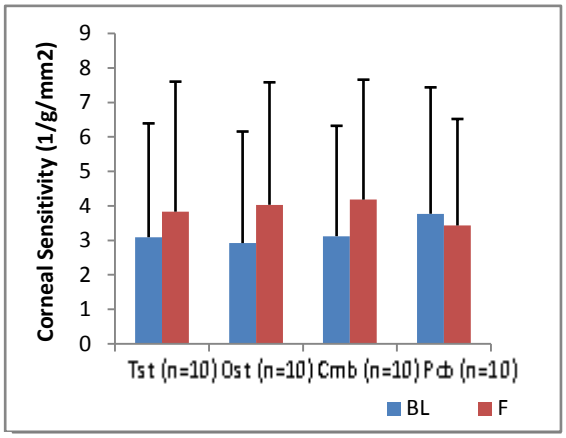
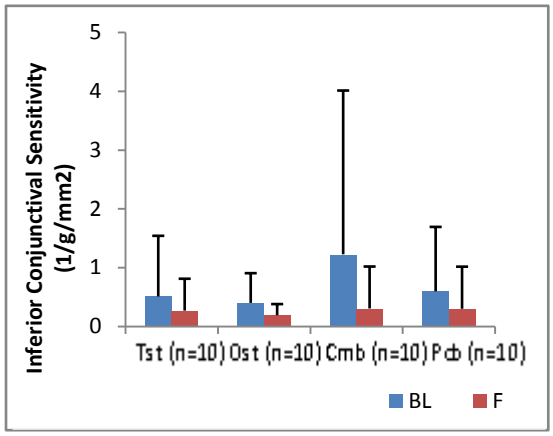


Figure 5.36 Mean group inferior conjunctival sensitivity by visit



5.6.4.5 Clinical Signs

5.6.4.5.1 Tear Function

Tear function measurements are shown in figures 5.37 to 5.42. Tear volume (PRT) improved in group 3 (p=0.04) but reduced in group 4 (p=0.07) post treatment (Figure 5.37). However there were no significant differences in tear volume (Schirmer) (Figure 5.38), tear osmolarity (Figure 5.39) and NIBUT (Figure 5.40) post treatment.

Figure 5.37 Mean group of tear volume (PRT) by visit

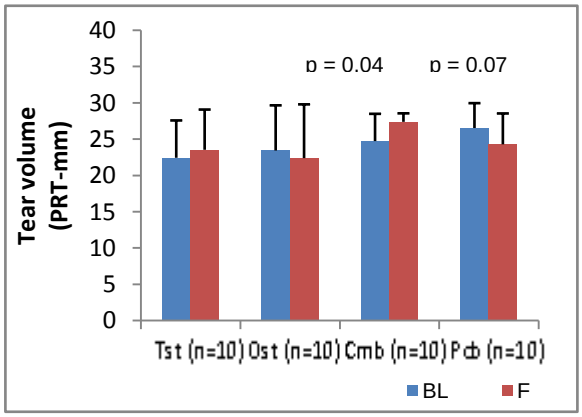


Figure 5.38 Mean group of tear volume (Schirmer) by visit

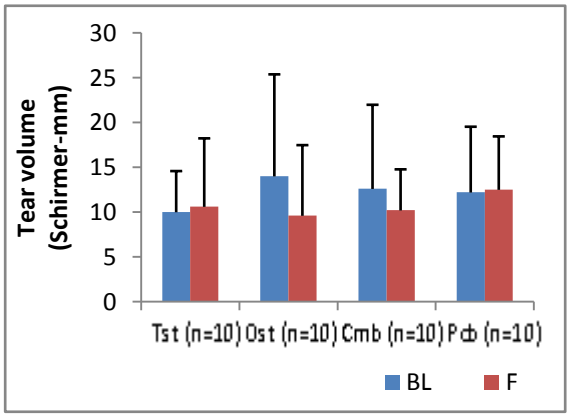


Figure 5.39 Mean group of tear osmolarity by visit

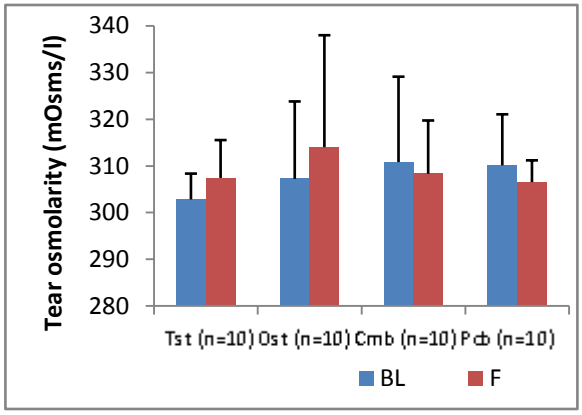
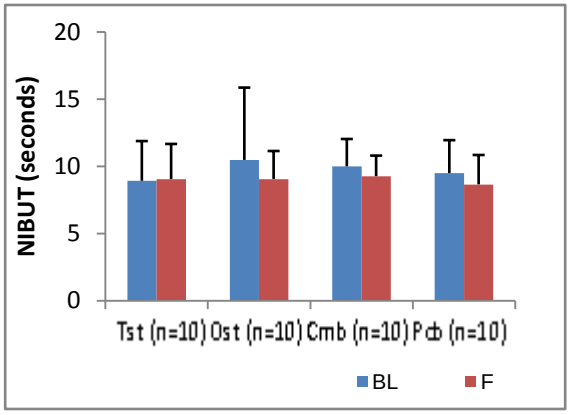


Figure 5.40 Mean group of NIBUT by visit



Ocular surface integrity assessments are shown in Figures 5.41 to 5.42. Corneal staining decreased in group 2 (p=0.06) but increased in group 1 (p=0.05) post treatment (Figure 5.41). Conjunctival staining reduced in groups 1 (p=0.09), 2 (p=0.02) and 3 (p=0.02) (Figure 5.42).

Figure5**.41** Mean group of corneal staining for each visit

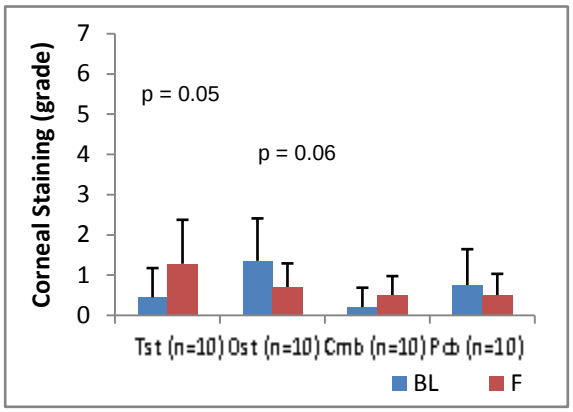
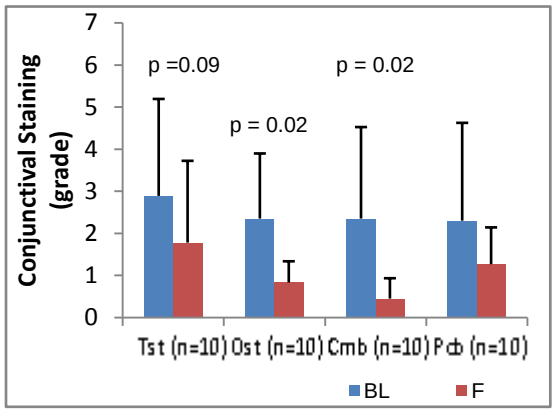


Figure 5.42 Mean group of conjunctival staining for each visit



5.7 Discussion

For the first time, transdermal hormones have been studied in a double-masked randomised placebo-controlled intervention study in a dry eye population. Compared with the placebo, there was less improvement in OCI dryness intensity with oestrogen treatment. Corneal staining was the only clinical sign showing a significant increase with intervention as recorded in both testosterone and the combined treatments, compared to placebo. The increase in serum oestradiol concentration and the ratio of oestradiol to androgen was significantly greater in the oestrogen and the combined treatment groups than in placebo, confirming the absorption of the hormone into the circulation.

5.7.1 **Oestrogen Treatment Group**

5.7.1.1 Effect of Intervention (Comparison with Placebo)

An increase in the dryness intensity in the oestradiol treatment group was significantly different to a reduction in this symptom in the placebo group. The worsening of dryness intensity with the increased oestradiol concentration is consistent with the study hypothesis**.** However, the profound improvement in the placebo group is unexpected and the responses to symptoms might be influenced by the Hawthorne effect (1920s-1930s). This effect is defined as "the phenomenon of altered behaviour or performance resulting from awareness of being a part of an experimental study" (Campbell et al 1995). Armed with good expectation from the treatment and the awareness of being studied, the subjects might have felt "cured" from dry eye after the eight-week intervention. In addition, the placebo effect where the true decrease in pain intensity occurred due to the release of analgesic substances within the brain parenchyma (Berthelot et al 2011) might have affected the subjects' responses to the ocular symptom questionnaires presented.

5.7.1.2 Effect of Intervention (Changes over time: Baseline versus Final)

A significant increase in the serum oestradiol concentration in the oestrogen treatment was demonstrated on the final visit.

The oestrogen treatment group demonstrated greater staining than the testosterone treatment group at baseline. After eight weeks of intervention, corneal staining and conjunctival staining improved in contrast to the association between oestradiol concentration and the worsening of corneal staining in the same population presented in Chapter 4 (without treatment). The latter finding was ascribed to the effect of oestrogen in promoting the gene expressibility of inflammatory cytokines and matrix metalloproteinases (MMPs) at the respective oestrogen receptors on the cornea (Suzuki et al. 2009). This might have led to corneal surface damage (Feenstra & Tseng 1992)and hence compromised surface integrity. In contrast, oestradiol was demonstrated to suppress the

expressibility and production of hyperosmolarity-induced pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) in human corneal epithelial cells (Wang et al 2012). These cytokines act as toxic agents toward the corneal epithelia, both by a direct osmotic mechanism and by mediated inflammatory activity which may lead to ocular surface damage (Rolando & Zierhut 2001) and hence corneal staining. Therefore, suppression of the pro-inflammatory cytokines by oestrogen might decrease disruption of the corneal surface and corneal staining as observed with oestrogen therapy.

After eight weeks of treatment, a significant improvement in the sexual domain score of the MENQOL and a reduction in OSDI score were reported with oestrogen. Among the questions addressed in the sexual domain was the presence of vaginal dryness during intercourse(Hilditch et al 1996). The prevalence of vaginal dryness (40.4%) was almost similar to eye dryness (42%) in a comparison study between vaginal symptoms and other climacteric symptoms(Takamatsu et al 2001). In addition, vaginal dryness was part of the validated climacteric symptoms, previously demonstrated to be positively associated with eye dryness(Stadberg et al 2000). Although oestradiol is mediated through two distinct intracellular receptors that share a similar binding affinity profile; ERα and ERβ, tissue localisation studies have revealed distinctly different expressibility patterns for each receptor (Chang et al 2008, Hall et al 2001, Kuiper et al 1997, Pearce & Jordan 2004).However, a higher concentration of oestrogen has improved the scores of vaginal dryness and OSDI post treatment, which was possibly due to the similar type of oestrogen receptors being activated on the vaginal and ocular surface tissues respectively. There were no significant changes in the other MENQOL domain scores which may be due to the mildness of the systemic symptoms (Haines et al 2005) in these study population.

Four randomised controlled double-masked published studies have investigated the effects of oestradiol therapy on dry eye in postmenopausal women (Table 5.8). An improvement in dry eye symptoms and tear function in post-menopausal women with dry eye and symptomatic females was demonstrated with oestrogen therapy (Akramian et al 1998, Sator et al 1998, Scuderi et al 2012) although differences existed in the delivery route of oestradiol and the duration of the study. Phytoestrogens used in one of these studies (Scuderi et al 2012) may have enhanced the androgenic effect with the elevation in

testosterone level (Gunnarsson et al 2009)which allows the improvement in signs of dry eye. The improvement in symptoms was however transient, which reappeared during the washout period in the cross-over design study (Scuderi et al 2012). The duration of the other studies were four months (Sator et al 1998) and one week (Akramian et al 1998)which might be insufficient to significantly determine longer term potentially adverse effects (Schaumberg et al 2001). In the current study, after eight weeks of oestrogen treatment, improvement in the ocular symptoms, corneal and conjunctival staining, and sexual domain scores were recorded. Eight weeks of treatment was the maximum duration allowed to observe the effect of testosterone and avoid the adverse effects of the treatment.

Table 5.5 Randomised placebo-controlled Studies on Hormone Replacement Therapy in Postmenopausal Women with Dry Eye

Investigator/ Study Design	Subjects	Type of HRT	Route of HRT Delivery	Duration of HRT usage	Significant Dry Eye Related Changes	
Sator 1998 (RCT)	1) 42 DE 2) 42 DE as controls	E2 placebo	Topical	4 months	↓symptoms ↑Schirmer in E2 receivers	
Akramian1998 (RCT)	1) 11symptomatic 2) 11symptomatic (45-65years in both groups)	E2 Placebo	Topical	one week	↓symptoms ↑ Schirmer&TBUT in E2 receivers	
Piwkumsribonruang 2010 (RCT)	1) 21 DE 2) 21 DE controls	E2+Pro placebo	Transdermal + oral	3 months	No significant changes in symptoms, Schirmer and TBUT	
Scuderi 2012 (RCT Crossover)	1) 66 DE	Phytoestrogen /placebo	Oral	30 days	↓OSDI ↑ Schirmer&TBUT, ↓tear osmolarity	
Current Study	1) 10 DE 2) 10 DE 3) 10 DE 4) 10 DE	E2 Testosterone E2+ Testosterone Placebo	Transdermal	8weeks/ 56 days	(Final – Baseline) vs placebo >worsening of symptoms with E2 > corneal staining with testosterone and combined treatments	Baseline vs Final ↑tear volume with combined treatment ↓symptoms with testosterone and combined treatment ↓corneal staining with oestradiol but ↑ with testosterone ↓conjunctival staining with all treatments

5.7.2 Testosterone treatment group

5.7.2.1 Effect of Intervention (Comparison with Placebo)

Testosterone treatment has no effect on symptoms or signs when compared to placebo which might be due to insufficient change in the serum concentration of androgen post treatment. This might be due to the low dosage of treatment [5 mg/daily (35mg/week)], which was selected to minimise side effects in the current study. There is no recommended dosage of testosterone treatment specifically for women, although in men a dosage (125 mg/week)was considered to be the best trade-off of beneficial and adverse effects on fat-free mass and muscle strength (Bhasin et al 2005). In addition, the current study used a 1%concentration of testosterone transdermal cream (systemic) instead of 3% as used by Connor (2003) (local application around the eye) to treat dry eye. Furthermore, in another intervention study, a Psychological General Well-Being Index increased significantly with the daily supply of 300 µg instead of 150 µg of testosterone (Shifren et al 2000) in postmenopausal women. This finding indicates that the effect of treatment might depend on the concentration, volume and administration route (systemic versus local) of the hormones supplied.

Apart from the hormone concentration, the analyte selected may also influence the observed effect of treatment. Although the androgen metabolites are recommended as a marker of androgenic activity, subjects' serum free testosterone levels may be measured to understand the consequences of testosterone treatment on free androgen. In addition Dihydrotestosterone (DHT) could also be utilised as a marker since DHT cannot be converted by the enzyme aromatase to oestradiol and hence may distinguish between the effects of testosterone caused by the androgen-receptor interaction and those caused by testosterone's conversion to oestradiol and subsequent binding to oestrogen receptors (Swerdloff & Wang 1998).

In contrast to study hypothesis, the worsening of corneal staining was recorded when compared to the placebo. Nevertheless, corneal staining might not be considered a specific sign of dry eye since the sign might be caused by other factors such as short-term and, more often, the long-term use of topical medications toxicity (Wilson 1979). In addition, corneal staining might not be considered a very sensitive measure, as it is detected in only 10% of dry eyes (Schiffman et al 2000).

5.7.2.2 Effect of Interventionover time: (Baseline versus Final)

Conjunctival staining was less in the final visit compared to baseline although the androgen levels were consistent throughout the intervention period. In dry eye, conjunctival surface damage has been proposed to precede the corneal damage (Yokoi & Kinoshita 1998) and the temporal conjunctival staining is considered an important non-invasive test to distinguish primary Sjögren syndrome from non-Sjögren keratoconjunctivitis sicca (Caffery et al 2010b). In addition, conjunctival staining was demonstrated as a reliable predictor of symptoms even in a normal-to-mild dry eye subjects as described in Section 3.6.2. It is speculated that healthy regulation of the lacrimal functional unit leads to sufficient tear supply that will lubricate the ocular surface and result in reduced conjunctival staining and therefore reduced symptoms. The significantly more number of years since menopause in the placebo group compared to the combined treatment group at baseline did not affect the androgen concentrations since there was no placebo effect on this measurement in the intervention over time analysis. Furthermore, years since menopause was not among the predictors of sex hormone concentration in postmenopausal women (Cauley et al 1989) and was not associated with symptoms as described in Chapter 4.

Testosterone treatment was not able to affect ocular surface sensitivity in the current study although a direct effect of these sex hormones on their cognate receptors was proposed (Bereiter et al 2005, Brown et al 1996, Romano et al 1988). The mean concentration of 3α-diol G in the testosterone treatment group was still lower (5.5 ± 11.5) ng/mL than in males (7.0 ± 5.9) ng/mL as described in Chapter 3, whose testosterone concentration was positively associated with an improvement in the corneal sensitivity. The lower concentration of androgen might have prevented the improvement in corneal sensitivity in this dry eye group.

5.7.3 Combined Treatment

5.7.3.1 Effect of Intervention (Comparison with Placebo)

In contrast to the hypothesis, the worsening of corneal staining was recorded in the combined treatment compared to the placebo. These findings were consistent with the findings in the testosterone treatment group (see Section 5.7.2.1).

5.7.3.2 Effect of Intervention (Changes over time: Baseline versus Final)

The testosterone and combination treatments were able to alleviate symptoms as previously reported (Scott et al 2005) and this outcome supports one of the hypotheses. The antagonistic characteristic of both oestrogen and testosterone treatment as described in Section 1.2.2 might be the source of the improvement in symptoms, tear volume and conjunctival staining in this group.

5.8 Study Limitations and Considerations

Patch treatment may be preferable with an improvement in dose control, patient acceptance, and compliance compared with the semisolid formulations (Brown et al 2006).

Significant differences in corneal staining at baseline might affect the treatment results. Corneal staining was significantly higher in the oestrogen treatment group than the testosterone treatment group due to the outliers.

A fasting blood collection that should have been performed before noon was not included in the study procedure and might have affected the hormone concentration results as described in 4.7.4.

With regards to other variables that were not significantly affected by the hormone treatments, a larger sample size should be considered to allow a better observation of these effects. Post hoc sample size calculation indicated that 114 subjects (28 in each treatment group) would allow the detection of 0.8 units of OCI dryness intensity symptom score at a 5% level of significance for a power of 80%.OCI dryness intensity was chosen as the basis of sample size calculation for future study since the variable has shown a significant change with oestradiol treatment in the current study.

5.9 Conclusion

Sex hormone levels may not affect dry eye symptoms in this population conclusively. The transdermal androgen and/or oestrogen treatment did not affect the symptoms in postmenopausal women with dry eye as hypothesized. It is difficult to draw conclusions from this intervention study with the unexpected profound improvement in the placebo group, where the responses to the symptoms might be influenced through the Hawthorne

and the placebo effects. Therefore the placebo effect should be considered in an interventional study.

Testosterone and combination between oestrogen and testosterone treatments only affected ocular surface staining and not symptoms.

With the presence of unexpected changes in corneal staining in females, it may be helpful to ask about their menstrual cycle.

Study measurements should be free from significant differences between groups at baseline to avoid confusion over the actual effect of intervention.

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CHAPTER 6

THESIS SUMMARY AND RECOMMENDED FUTURE STUDIES

6.1 Thesis Summary

This thesis investigated the relationship between dry eye and sex hormone levels. The aims of this investigation were firstly, to identify the relationships between levels of circulating sex hormones and ocular surface sensitivity and dry eye symptoms and signs in normal-to-mild and moderate dry eye populations. Secondly, the thesis aimed to examine the effects of sex hormone treatments on these variables in a homogenous population of postmenopausal women with dry eye.

To achieve the first aim, a study was performed in a sample of a normal-to-mild dry eye population of both genders, which also allowed the impact of gender on these study variables being simultaneously investigated. The second study involved postmenopausal women with moderate dry eye where subjects with self-reported symptoms and previous diagnoses were enrolled. In these studies, 3α-diol G in the circulation represented the level of testosterone at the peripheral site (Labrie et al 2006, Labrie et al 2003), allowing associations between this androgen metabolite and the local levels of symptoms to be tested.

In the first study, dry eye symptoms and signs were positively associated with oestradiol and ratio of oestradiol to androgens in females but negatively associated with androgens in males in a sample of a normal-to-mild dry eye population. In addition, a higher tear volume was demonstrated in males. The effects of androgen and oestrogen on dry eye symptoms and signs were different between genders which might be due to the differences in hormone concentrations, gender-specific regulation of genes or in the number of hormone receptors present on the ocular surface (Rocha et al 1993, Sullivan et al 1996, Sullivan et al 1984, Sullivan et al 2009). These findings indicated the gender-based impact on dry eye symptoms and signs.

In the second study, for the first time, transdermal hormones have been studied in a double-masked randomised placebo-controlled intervention study in a dry eye population. Compared with the placebo, the relative worsening of dryness intensity symptoms with the increased oestradiol concentration was consistent with the study hypothesis. The profound improvement in the placebo group was unexpected and the responses to symptoms might be influenced by the Hawthorne and the placebo effects (Campbell et al 1995, Berthelot et al 2011).

The effect of sex hormone levels on the ocular surface sensitivity and menopausal systemic symptoms were also investigated, adding to the uniqueness of this thesis. The featured novel finding was the potential of ocular surface sensitivity being affected by sex hormones. In normal to mild dry eye subjects, a higher level of free testosterone and 3α-diol G were associated with increased corneal sensitivity in males. The positive significant association between central corneal sensitivity and free testosterone and 3α-diol G supports the hypothesis that a sufficient level of androgen (Labrie et al 2003) at the ocular sites is necessary to maintain normal homeostasis and a sufficiently lubricated and healthy ocular surface (Mathers 2000, Stern et al 2004, Sullivan et al 2000). Ocular surface sensitivity may be affected in dry eye by the androgen and oestrogen hormone-receptor activation on the ocular surface or indirectly through the neural feedback loop, linking the lacrimal gland and ocular surface (Mathers 2000, Stapleton et al 2013). This observation might occur only in males in the current study due to the higher concentration of androgen in males relative to females. Adding to the interesting findings above, the inferior conjunctival sensitivity was among the significant predictors of symptoms, revealing the importance of ocular surface sensitivity as an important dry eye clinical indicator.

The literature is equivocal on whether an increase of symptoms is associated with either hyper- or hypo-sensitivity changes on the ocular surface (Adatia et al 2004, Barboza et al 2008, Belmonte et al 1999, Benítez-del-Castillo et al 2007, Bourcier et al 2005, De Paiva & Pflugfelder 2004, Han et al 2010, Situ et al 2008b, Toker & Asfuroglu 2010, Tuisku et al 2008, Versura et al 2006, Xu et al 1996). This uncertainty provides the opportunity for further research studies to be undertaken.

Although corneal sensitivity was featured as a significant variable, with significant positive associations with androgens in the preliminary study, insufficient level of 3α-diol G in the postmenopausal women with moderate dry eye might have prevented significant

association between the hormone and corneal sensitivity from occurring (chapter 4). Hence, we may suggest that corneal sensitivity changes may be affected by the androgen but not oestradiol level. However, even with androgen (testosterone) supplement in the clinical trial (chapter 5), there was also no corneal sensitivity change in groups with an increase in corneal staining. This could mainly be due to the absence of significant change in the androgen level post treatment, as a result of the insufficient concentration, volume and administration route (systemic versus local) of the hormones supplied. Furthermore, corneal sensitivity and staining were not significantly associated with each other in this moderate dry eye population.

In this thesis, conjunctival sensitivity has been revealed to be a predictor of dry eye symptoms in females. Therefore, it is recommended that clinicians perform the conjunctival sensitivity measurement with a Cochet-Bonnet, as carried out in the studies in this thesis. The recorded measurement should be monitored during visits since a compromised conjunctival sensitivity may indicate a dry eye condition.

In postmenopausal women with moderate dry eye, systemic symptoms were included among the study variables as stated above. Dry eye symptoms have also been associated with systemic symptoms (Shimmura et al 1999); and vaginal dryness (Stadberg et al 2000) and these symptoms were among the questions queried in the physical and sexual domains of the Menopause-Specific Quality of Life (MENQOL) questionnaire (Hilditch et al 1996, Hilditch et al 2008). However, systemic symptoms were surprisingly not affected by the circulating sex hormone levels and none of the MENQOL domain scores were associated with ocular symptoms. Such lack of associations might be due to the subjects having only mild systemic symptoms (Haines et al 2005), moderate dry eye symptoms, as well as, the positive acceptance of the deterioration in physical health (Smeeth & Iliffe 1998) and possibly ocular symptoms with age, among the study subjects.

In this moderate dry eye population, the associations between oestradiol, androgens and the ratio of oestradiol to androgens and dry eye symptoms, systemic symptoms and the majority of the clinical signs were not consistently demonstrated. In addition, sex hormones were not able to consistently affect symptoms of dry eye. In this population, the only two significant associations recorded were between greater corneal staining and a higher level of both oestradiol and ratio of oestradiol to 3α-diol G, but not 3α-diol G. The subjects'

oestradiol concentration range was lower, than the expected oestradiol range in postmenopausal women, which may have led to the lack of other expected significant associations. In addition, the concentration of 3α-diol G and DHEA-S was within the normal range, instead of lower (as predicted in postmenopausal women with dry eye).

The severity of this disease might also influence the lack of associations between sex hormone concentrations and most of the study variables. Most of the previous studies which demonstrated associations between sex hormones and dry eye signs were performed in subjects with severe dry eye (Gagliano et al 2014, Scuderi et al 2012) unlike the present study, which included subjects with less severe disease. We can conclude that the severity of dry eye; lower concentration of oestradiol and consistent concentration of androgen might have led to the lack of significant associations between sex hormones and symptoms and most of the clinical signs in this population. This normal range of androgens might still be sufficient to regulate the lacrimal and systemic symptoms.

Other factors, which could have been considered in this study, would include the possible impact of diurnal variability on the sex hormone concentrations. In addition,the DHEA-S concentration in the current study may have also been affected since the plasma samples were stored for almost a year where the hormone was shown to decrease approximately by 5% yearly in storage (Hankinson et al 1995).

Age also affected dry eye. Significant associations between age and reductions in oestradiol and androgens levels; and in the number of patent glands but increased tear osmolarity were recorded in the normal-to-mild dry eye population. Therefore, it is important to adjust for age and gender when considering the effects of sex hormone levels on dry eye.

The contribution of androgen produced by the postmenopausal ovary to the circulating pool of androgen is controversial. Among oophorectomised women, testosterone levels were not affected by age and were 40-50% lower than those in intact women throughout the 50-89 year age range (Fogle et al 2007, Laughlin et al 2000). However the enzymes for androgen biosynthesis were either absent or present in very low amounts in postmenopausal ovary (Couzinet et al 1989). In the present study, the mean of years since menopause was relatively high at 12.9 ± 6.6 years. However, years since menopause was not among the predictors of sex hormone concentration in postmenopausal women (Cauley

et al 1989) and was not associated with symptoms in the current study. Therefore it cannot be concluded that the normal range of 3α-diol G and DHEA-S concentration was due to the contribution of the hormone by the ovaries.

Meibomian gland expressibility and lid margin vascularisation assessments were consistent predictors of symptoms in the postmenopausal women who were recruited based on self-reported dry eye symptoms. Meibomian gland secretion limits evaporative tear loss, provides a barrier function at the lid margin, supplies lubrication during blinking, and maintains an optically smooth ocular surface (Nichols et al 2011). The majority of evaporative dry eye cases are due to compromised meibomian gland function (Foulks & Bron 2003, Bron & Tiffany 2004a, Bron & Tiffany 2004b). Physiological changes to the gland orifices due to aging (Den et al 2006, Hykin & Bron 1992) may reduce the function of meibomian gland stated above and hence lead to evaporative dry eye which is usually accompanied by symptoms of dry eye. Based on this multivariate analysis outcome, more emphasis should be given to meibomian gland dysfunction as a cause of dry eye symptoms in postmenopausal women.

A comparison between postmenopausal women with dry eye (subjects of the study in Chapter 4) and without dry eye (subjects of the study in Chapter 3) showed no significant differences in oestradiol, DHEAS and 3α-diol G levels. However, the study was not designed with sufficient power to test this particular hypothesis but this would be relevant to explore in future studies.

The potential predictors of symptoms in both populations have also been identified. The importance of the relationship between sex hormones and dry eye symptoms was confirmed where oestradiol, and the ratio of oestradiol to androgens emerged as factors in the final models of the multivariate analysis. However, sex hormones had less impact on dry eye symptoms than other factors in both normal-to-mild and moderate dry eye populations. The regression analysis revealed that symptoms were predicted by conjunctival sensitivity and staining; and NIBUT in the normal-to-mild dry eye population. This finding suggests that habitual physiological changes in the sex hormone concentration for instance during the menstrual cycle or menopausal phases (pre, peri or postmenopausal) in a normal-to-mild dry eye population may not affect dry eye symptoms.

However, sex hormone levels are important to be considered especially in females since a higher concentration of circulating oestradiol is significantly associated with the worsening of corneal staining, as revealed in this thesis. Therefore, it is important to ask the women who come to the clinic whether they are on hormone medication or therapy during history taking. Clinicians should be cautious of the effects of such medication on clinical signs such as corneal staining.

The presence of oestradiol and the ratio of oestradiol to androgen in the final models of the regression analysis in both populations suggest the importance of relationship between sex hormones and dry eye symptoms. Therefore a double-masked randomised placebo-controlled eight week pilot intervention study was performed to examine the effect of oestrogen, testosterone and their combination treatment on symptoms and signs of dry eye in this population. To confirm the absorption of the hormone into the circulation in an intervention study, the sex hormone concentrations were measured prior to and immediately after the treatment duration.

Apart from the possible influence of the Hawthorne and the placebo effects (Campbell et al 1995, Berthelot et al 2011) demonstrated in this study, there are a few interesting significant findings in individuals receiving oestrogen treatment when the baseline and final measurements were compared. Firstly, corneal staining and conjunctival staining improved with oestrogen which is speculated to be due to the suppression of the pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) in human corneal epithelial cells (Wang et al 2012). These cytokines act as toxic agents toward the corneal epithelia, both by a direct osmotic mechanism and by mediated inflammatory activity which may lead to ocular surface damage (Rolando & Zierhut 2001) and hence corneal staining. Therefore, suppression of the pro-inflammatory cytokines by oestrogen might decrease disruption of the corneal surface and corneal staining as observed with oestrogen therapy.

Secondly, a significant improvement in the sexual domain score of the MENQOL and OSDI score were reported. Vaginal dryness was one of the items in the sexual domain scale and was also among the validated climacteric symptoms positively associated with eye dryness (Stadberg et al 2000). A higher concentration of oestrogen has improved the scores of vaginal dryness and OSDI post treatment, which was possibly due to the similar type of oestrogen receptors being activated on the vaginal and ocular surface tissues respectively.

Testosterone and combination treatment had no effect on symptoms or signs when compared to placebo since 1% of testosterone used might presumably be the low dosage of treatment. Apart from hormone concentration, Dihydrotestosterone (DHT) might have been a better analyte to measure in future studies since DHT cannot be converted by the enzyme aromatase to oestradiol and hence may distinguish between the effects of testosterone caused by the androgen-receptor interaction and those caused by testosterone's conversion to oestradiol and subsequent binding to oestrogen receptors (Swerdloff & Wang 1998).

Contrary to the hypothesis, testosterone and the combined treatment caused increased corneal staining when compared to the placebo. However, corneal staining might not be considered a very sensitive measure, as it is detected in only 10% of dry eyes (Schiffman et al 2000).

Compared with the baseline measurement, treatment with testosterone and the combined treatment improved both conjunctival staining and symptoms, although this was not significant when adjusting for the effect of the placebo.

We can conclude that transdermal treatment with oestrogen causes worsening of the intensity of ocular dryness, which is consistent with the study hypothesis. Testosterone and combination between oestrogen and testosterone treatments only affected ocular surface staining and not symptoms. The effect of treatment might depend on the concentration of the hormones supplied.

The combination oestrogen-progesterone therapy was reported to improve symptoms and signs of dry eye (Affinito et al 2003, Altintaş et al 2004, Coksuer et al 2011, Guaschino et al 2003, Jung et al 2010, Kuscu et al 2003, Uncu et al 2006). This improvement is may be due to the ability of progesterone to prevent the impact of oestrogen alone in worsening the dry eye condition (Schaumberg et al 2001). However there was no investigation of the effect of progesterone alone on dry eye. Therefore, Chapter 3 described an exploratory study that allowed us to investigate the potential associations between progesterone and ocular surface sensitivity and dry eye symptoms and signs in a normal to mild dry eye population. Nevertheless, there were no significant associations between progesterone and the other variables. Hence, progesterone was not included in the studies in chapters 4 and 5. Furthermore, the intervention study in this thesis might have limited its focus only on

testosterone and oestradiol. It is also possible that the study designs were not adequate to rule out a role for progesterone, given that progesterone levels were only measured in a normal population. Further investigation should be performed in a more severe dry eye population. This may perhaps lead to the identification of significant associations between progesterone level and dry eye symptoms and signs. Combined oestrogen/progesterone therapy should also be included in the intervention study on post menopausal dry eye population following the identification of these significant associations.

The thesis findings may have helped to clarify some issues regarding sex hormones but was not able to resolve questions such as the difference of sex hormone levels between the dry eye and non-dry eye postmenopausal women and that one of the most unexpected things was the strong placebo effect. In addition, circulating sex hormone levels may not affect dry eye symptoms.

6.2 Recommendation for Future studies

The study reported in chapter 5 was designed and approved by the local ethics committee as a pilot study only, such that 10 subjects were treated with each treatment of testosterone, oestrogen and the combination between testosterone and oestrogen. A larger sample size may allow further exploration of the effects of oestrogen and testosterone and their combination on dry eye symptoms and signs. Since DHEA-S was associated with an improvement in tear osmolarity in the normal-to-mild dry eye population, treatment with this androgen metabolite could be considered as a next step to identify a hormone based treatment for dry eye.

Although androgen metabolites discussed here are recommended as a marker of androgenic activity, subjects' serum free testosterone levels may be measured to understand the consequences of testosterone treatment on free androgen. In addition, Dihydrotestosterone (DHT) should be measured since DHT may distinguish between the effects of testosterone caused by the androgen-receptor interaction and those caused by testosterone's conversion to oestradiol and subsequent binding to oestrogen receptors (Swerdloff & Wang 1998).

Morning collection of fasting blood is preferable to minimise the change in hormone levels associated with eating and to avoid circadian variation. The plasma level of DHEA-S in

postmenopausal women decreases over time with storage, with a 25% decrease in 5 years of storage in liquid nitrogen freezers (Hankinson et al 1995). Therefore it is appropriate for the storage of plasma or serum to not exceed a year to avoid depletion in hormone levels over time.

A higher concentration of treatment (testosterone) may allow the actual effect of testosterone on dry eye symptoms and signs to be demonstrated. The lack of effect of testosterone treatment on serum androgen may either relate to inadequate dosing or measurement of a less than optimal analyte, the route and type of treatment, where patch treatment or topical therapy is preferable with an improvement in dose control, patient acceptance, and compliance compared with the semisolid formulations (Brown et al 2006).

Although ELISAs have the advantage of being technically simple, rapid, relatively inexpensive and allowing high throughput in measuring androgen and oestrogen levels, the hormone concentration is often overestimated, results and reference intervals are not standardised or not well documented in different populations (Rosner et al 2007). The oestradiol measured in the postmenopausal women with dry eye was lower rather than higher than the documented level in the normal postmenopausal women.

The most widely used methods for measuring oestrogen in postmenopausal women are RIA and ECLIA (Blair 2010) although hormone levels in postmenopausal women are close to the limit of detection for these assays (Cauley et al 1991, McShane et al 1996). More sensitive RIA coupled with liquid chromatography currently provides the most sensitive and best validated immunoassay method for oestrone and oestradiol in serum in postmenopausal women (Blair 2010). However, this technique is costly and time consuming for the extraction and purification processes. Mass spectrometry is another technique in which multiple steroids can be measured in the same sample aliquot, offers a highly accurate hormone concentration reading if properly validated, and the technique is generally comparable with RIA after extraction and chromatography. However, mass spectrometry is relatively expensive, time consuming, has a limited throughput, and the organic solvents used in the process require special facilities and waste disposal(Rosner et al 2007).

Meibomian gland dysfunction was revealed as a consistent predictor of symptoms in the postmenopausal women who were recruited based on self-reported dry eye symptoms and

not on meibomian gland assessment scores. It is clearly important to address dry eye in this group which may also allow the impact of other factors on symptoms to be evaluated.

Studies with sufficient power to determine associations between age and symptoms and signs; and associations between androgen concentrations and symptoms and signs in premenopausal women should be carried out to further understand the effect of age and gender on dry eye.

6.3 Conclusion

This thesis evaluated symptoms and signs of dry eye and circulating sex hormone levels in several population groups and established the impact of administration of transdermal sex hormones on dry eye in postmenopausal women.

Symptoms and clinical signs of dry eye were consistently associated with age and gender in a normal-to-mild dry eye population. Tear osmolarity increased and the number of patent meibomian glands decreased with age. Females reported slightly but not statistically greater symptoms on all scales tested ($p<0.1$) and lower tear volume compared with males ($p<0.05$). To reiterate the previous studies which proved that habitual levels of circulating androgen and oestrogen had different impacts on genders which may be due to the differences in hormones concentrations, gender-specific regulation of genes and the number of hormone receptors present on the ocular surface.

Significant associations between the circulating level of oestradiol, testosterone and the ratio of oestradiol to androgens and dry eye symptoms and signs were found in a normal-to-mild dry eye population of both genders. However there were no relationships between circulating progesterone and dry eye symptoms and signs. This adds information to the currently limited literature on associations between circulating sex hormones and clinical findings in dry eye. Sex hormones had less impact on dry eye symptoms than other factors in both normal-to-mild and moderate dry eye groups. More emphasis should be given to meibomian gland assessment in the process of determining dry eye symptoms especially in postmenopausal women.

The worsening of dryness intensity with oestradiol treatment is consistent with the study hypothesis but the relationship between habitual oestradiol level and dryness symptoms could not be confirmed in multivariate analysis. Testosterone and the combination of

oestrogen and testosterone treatments only affected ocular surface staining and not symptoms.

REFERENCES

Australian Medical Research Council (2004). Hormone Replacement Therapy For Women At Or After The Memopause: A Comprehensive Literature Review. Retrieved on 8 June 2010 from http://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/wh34_complete.pdf.

Acosta MC, Alfaro ML, Borrás F, Belmonte C, Gallar J. 2006. Influence of age, gender and iris color on mechanical and chemical sensitivity of the cornea and conjunctiva. Experimental Eye Research 83: 932-38

Adatia FA, Michaeli-Cohen A, Naor J, Caffery B, Bookman A, Slomovic A. 2004. Correlation between corneal sensitivity, subjective dry eye symptoms and corneal staining in Sjogren's syndrome. Canadian Journal of Ophthalmology. 39: 767-71

Affinito PS, Attilio DS, Di Carlo C, Sammartino A, Tommaselli GA, et al. 2003. Effects of hormone replacement therapy on ocular function in postmenopause. Menopause 10: 482-87

Afonso AA, Monroy D, Stern ME, Feuer WJ, Tseng SCG, Pflugfelder SC. 1999. Correlation of tear fluorescein clearance and schirmer test scores with ocular irritation symptoms, Historical image. Ophthalmology 106: 803-10

Akramian J, Wedrich A, Nepp J, Sator M. 1998. Estrogen Therapy in Keratoconjunctivitis Sicca In Lacrimal Gland, Tear Film, and Dry Eye Syndromes 2, ed. D Sullivan, D Dartt, M Meneray, pp. 1005-09: Springer US

Allen AL, McCarson KE. 2005. Estrogen increases nociception-evoked brain-derived neurotrophic factor gene expressibility in the female rat. Neuroendocrinology 81: 193-99

Altintaş Ö, Caglar Y, Yüksel N, Demirci A, Karabaş L. 2004. The effects of menopause and hormone replacement therapy on quality and quantity of tear, intraocular pressure and ocular blood flow. Ophthalmologica 218: 120-29

Amandusson Å, Hallbeck M, Hallbeck A-L, Hermanson O, Blomqvist A. 1999. Estrogen-induced alterations of spinal cord enkephalin gene expressibility. Pain 83: 243-48

Anasti JN, Kalantaridou SN, Kimzey LM, Defensor RA, Nelson LM. 1998. Bone loss in young women with karyotypically normal spontaneous premature ovarian failure. Obstetrics & Gynecology 91: 12-15

Arita R, Itoh K, Inoue K, Amano S. 2008. Noncontact infrared meibography to document age-related changes of the meibomian glands in a normal population. Ophthalmology 115: 911-15

Arita R, Itoh K, Maeda S, Maeda K, Furuta A, et al. 2009. Proposed Diagnostic Criteria for Obstructive Meibomian Gland Dysfunction. Ophthalmology 116: 2058-63

Asbell PA, Stapleton FJ, Wickström K, Akpek EK, Aragona P, et al. 2011. The International Workshop on Meibomian Gland Dysfunction: Report of the Clinical Trials Subcommittee. Investigative Ophthalmology & Visual Science 52: 2065-85

Auw-Haedrich C, Feltgen N. 2003. Estrogen receptor expressibility in meibomian glands and its correlation with age and dry-eye parameters. Graefe's Archive for Clinical and Experimental Ophthalmology 241: 705-09

Avis NE, Stellato R, Crawford S, Bromberger J, Ganz P, et al. 2001. Is there a menopausal syndrome? Menopausal status and symptoms across racial/ethnic groups. Social Science & Medicine 52: 345-56

Bachmann G, Bancroft J, Braunstein G, Burger H, Davis S, et al. 2002. Female androgen insufficiency: the princeton consensus statement on definition, classification, and assessment. Fertility and Sterility 77: 660-65

Bancroft J, Sherwin BB, Alexander GM, Walker A. 1991. Oral contraceptives, androgens, and the sexuality of young women: II. The role of androgens. Archives of Sexual Behavior 20: 121-35

Barboza MNC, Barboza GNC, Melo GMd, Sato É, Dantas MCN, et al. 2008. Correlação entre sinais e sintomas de olho seco em pacientes portadores da síndrome de Sjögren (English version). Arquivos Brasileiros de Oftalmologia 71: 547-52

Begley C, Caffery B, Chalmers R, Mitchell G. 2002a. Use of the dry eye questionnaire to measure symptoms of ocular irritation in patients with aqueous tear deficient dry eye. Cornea 21: 664 - 70

Begley C, Caffery B, Nichols K, Mitchell G, Chalmers R. 2002b. Results of a dry eye questionnaire from optometric practices in North America. Advance Experimental Medicine Biology 506: 1009 - 16

Begley CG, Caffery B, Chalmers RL, Mitchell GL, Group DEIS. 2002c. Use of the Dry Eye Questionnaire to Measure Symptoms of Ocular Irritation in Patients With Aqueous Tear Deficient Dry Eye. Cornea 21: 664-70

Begley CG, Caffery B, Nichols KK, Chalmers R. 2000. Responses of Contact Lens Wearers to a Dry Eye Survey. Optometry & Vision Science 77: 40-46

Belmonte C, Acosta MC, Schmelz M, Gallar J. 1999. Measurement of corneal sensitivity to mechanical and chemical stimulation with a CO2 esthesiometer. Investigative Ophthalmology & Visual Science 40: 513-9

Belmonte C, Carmen Acosta M, Gallar J. 2004. Neural basis of sensation in intact and injured corneas. Experimental Eye Research 78: 513-25

Belmonte C, Garcia-Hirschfeld J, Gallar J. 1997. Neurobiology of ocular pain. Progress in Retinal and Eye Research 16: 117-56

Benítez-del-Castillo JM, Acosta MC, Wassfi MA, Díaz-Valle D, Gegúndez JA, et al. 2007. Relation between Corneal Innervation with Confocal Microscopy and Corneal Sensitivity with Noncontact Esthesiometry in Patients with Dry Eye. Investigative Ophthalmology & Visual Science 48: 173-81

Benitez-del-Castillo JM, del Rio T, Iradier T, Hernández JL, Castillo A, Garcia-Sanchez J. 2001. Decrease in Tear Secretion and Corneal Sensitivity After Laser In Situ Keratomileusis. Cornea 20: 30-32

Bereiter DA, Barker DJ. 1980. Hormone-induced enlargement of receptive fields in trigeminal mechanoreceptive neurons. I. Time course, hormone, sex and modality specificity. Brain Research 184: 395-410

Bereiter DA, Cioffi JL, Bereiter DF. 2005. Oestrogen receptor-immunoreactive neurons in the trigeminal sensory system of male and cycling female rats. Archives of Oral Biology 50: 971-79

Bereiter DA, Hirata H, Hu JW. 2000. Trigeminal subnucleus caudalis: beyond homologies with the spinal dorsal horn. Pain 88: 221-24

Berthelot J-M, Le Goff B, Maugars Y. 2011. The Hawthorne effect: stronger than the placebo effect? Joint Bone Spine 78: 335-36

Beyenburg S, Stoffel-Wagner B, Bauer J, Watzka M, Blümcke I, et al. 2001. Neuroactive steroids and seizure susceptibility. Epilepsy Research 44: 141-53

Bhasin S, Woodhouse L, Casaburi R, Singh AB, Mac RP, et al. 2005. Older men are as responsive as young men to the anabolic effects of graded doses of testosterone on the skeletal muscle. Journal of Clinical Endocrinology &Metabolism 90: 678-88

Bizzarro A, Valentini G, Martino GD, Daponte A, De Bellis A, Iacono G. 1987. Influence of Testosterone Therapy on Clinical and Immunological Features of Autoimmune Diseases Associated with Klinefelter's Syndrome. Journal of Clinical Endocrinology & Metabolism 64: 32-36

Blair I. 2010. Analysis of estrogens in serum and plasma from postmenopausal women: Past present, and future. Steroids 75: 297-306

Bland MJ, Altman DG. 1986b. Statistical Methods For Assessing Agreement Between Two Methods Of Clinical Measurement. The Lancet 327: 307-10

Blodi F. 1980. Eye problems of the elderly. Ophthalmologica 32:121-28

Bonini S, Mantelli F, Moretti C, Lambiase A, Bonini S, Micera A. 2007. Itchy-Dry Eye Associated with Polycystic Ovary Syndrome. American Journal of Ophthalmology 143: 763-71.

Bourcier T, Acosta MC, Borderie V, Borrás F, Gallar J, et al. 2005. Decreased Corneal Sensitivity in Patients with Dry Eye. Investigative Ophthalmology & Visual Science 46: 2341-45

Braunstein GD, Reitz RE, Buch A, Schnell D, Caulfield MP. 2011. Testosterone Reference Ranges in Normally Cycling Healthy Premenopausal Women. The Journal of Sexual Medicine 8: 2924-34

Brennan N, Efron N. 1989. Symptomatology of HEMA contact lens wear. Optometry & Vision Science 66: 834-38

Brinkmann A. 2001. Molecular basis of androgen insensitivity. Molecular and Cellular Endocrinology 179: 105-09

Bron AJ, Benjamin L, Snibson GR. 1991. Meibomian Gland Disease. Classification and Grading of Lid Changes. Eye 5: 395-411

Bron AJ, Evans VE, Smith JA. 2003. Grading of corneal and conjunctival staining in the context of other dry eye tests. Cornea 22: 640-50

Bron AJ, Tiffany JM, Gouveia SM, Yokoi N, Voon LW. 2004(a). Functional aspects of the tear film lipid layer. Experimental Eye Research 78: 347-60

Bron AJ, Tiffany JM. 2004(b).The contribution of Meibomian disease to dry eye.*Cornea 2: 149-64*

Bron AJ, Tripathi R, Tripathi B. 1997. Wolff's Anatomy of the Eye and Orbit 8th ed. London: Chapman & Hall Medical.

Brown EM, Pollak M, Seidman CE, Seidman JG, Chou Y-HW, et al. 1995. Calcium-Ion–Sensing Cell-Surface Receptors. New England Journal of Medicine 333: 234-40

Brown MB, Martin GP, Jones SA, Akomeah FK. 2006. Dermal and Transdermal Drug Delivery Systems: Current and Future Prospects. Drug Delivery 13: 175-87

Brown TJ, Scherz B, Hochberg RB, MacLusky NJ. 1996. Regulation of Estrogen Receptor Concentrations in the Rat Brain: Effects of Sustained Androgen and Estrogen Exposure. Neuroendocrinology 63: 53-60

Caffery B, Simpson T, Wang S, Bailey D, McComb J, et al. 2010a. Factor Analysis of the Clinical Characteristics of Primary Sjogren Syndrome. Optometry & Vision Science 87: 742-50

Caffery B, Simpson T, Wang S, Bailey D, McComb J, et al. 2010b. Rose Bengal Staining of the Temporal Conjunctiva Differentiates Sjögren's Syndrome from Keratoconjunctivitis Sicca. Investigative Ophthalmology & Visual Science 51: 2381-87

Campbell JP, Maxey VA, Watson WA. 1995. Hawthorne Effect: Implications for Prehospital Research. Annals of Emergency Medicine 26: 590-94

Cao D-Y, Ji Y, Tang B, Traub RJ. 2012. Estrogen receptor β activation is antinociceptive in a model of visceral pain in the rat. The Journal of Pain 13: 685-94

Cauley JA, Gutai JP, Kuller LH, Ledonne D, Powell JG. 1989. The Epidemiology Of Serum Sex Hormones In Postmenopausal Women. American Journal of Epidemiology 129: 1120-31

Cauley JA, Gutai JP, Kuller LH, Powell JG. 1991. Reliability and Interrelations among Serum Sex Hormones in Postmenopausal Women. American Journal of Epidemiology 133: 50-57

Cennamo GL, Del Prete A, Forte R, Cafiero G, Del Prete S, Marasco D. 2007. Impression cytology with scanning electron microscopy: a new method in the study of conjunctival microvilli. Eye 22: 138-43

Cermak JM, Krenzer KL, Sullivan RM, Dana MR, Sullivan DA. 2003. Is complete androgen insensitivity syndrome associated with alterations in the meibomian gland and ocular surface? Cornea 22: 516-21

Chabin V, Micevych P. 2005. Estrogen receptor-a mediates estradiol attenuation of ATP-induced Ca++ signaling in mouse dorsal root ganglion neurons. Journal of Neuroscience Research 81: 31-7

Chalmers RL, Begley CG, Caffery B. 2010. Validation of the 5-Item Dry Eye Questionnaire (DEQ-5): Discrimination across self-assessed severity and aqueous tear deficient dry eye diagnoses. Contact Lens and Anterior Eye 33: 55-60

Chang EC, Charn TH, Park SH, Helferich WG, Komm B, et al. 2008. Estrogen Receptors alpha and beta as determinants of gene expressibility: influence of ligand, dose, and chromatin binding. Molecular Endocrinology 22: 1032-43

Chang KC, Oh JY, In YS, Kim MK, Shin KC, et al. 2009. Preliminary Effects of Oral Uridine on the Ocular Surface in Dry Eye Patients. Journal of Korean Medical Science 24: 701-07

Chao C, Golebiowski B, Cui D, Stapleton F. 2013. Development of a Chinese version of the Ocular Comfort Index: preliminary validation findings. Investigative Ophthalmology & Visual Science 54: E Abstract 4351

Chao C, Golebiowski B, Stapleton F. 2014. The role of corneal innervation in LASIK-induced neuropathic dry eye. The ocular surface 12: 32-45

Chen S, Massaro-Giordano G, Pistilli M, Schreiber CA, Bunya VY. 2013. Tear Osmolarity and Dry Eye Symptoms in Women Using Oral Contraception and Contact Lenses. Cornea 32: 423-28

Chew CKS, Hykin PG, Jansweijer C, Dikstein S, Tiffany JM, Bron AJ. 1993. The casual level of meibomian lipids in humans. Current Eye Research 12: 255-59

Chia E-M, Mitchell P, Rochtchina E, Lee AJ, Maroun R, Wang JJ. 2003. Prevalence and associations of dry eye syndrome in an older population: the Blue Mountains Eye Study. Clinical and Experimental Ophthalmology 31: 229-32

Cho P, Yap M. 1993. Age, Gender, and Tear Break-Up Time. Optometry & Vision Science 70: 828-31

Clegg JP, Guest JF, Lehman A, Smith AF. 2006. The Annual Cost of Dry Eye Syndrome in France, Germany, Italy, Spain, Sweden and the United Kingdom Among Patients Managed by Ophthalmologists. Ophthalmic Epidemiology 13: 263-74

Cohen J. 1988. Statistical power analysis for the behavioral sciences 2nd Edition. Hillsdale, New Jersey: Lawrence Erlbaum Associates, Publishers.

Coksuer H, Ozcura F, Oghan F, Haliloglu B, Coksuer C. 2011. Effects of estradiol-drospirenone on ocular and nasal functions in postmenopausal women. Climacteric 14: 482-7

Conger AJ. 1974. A Revised Definition for Suppressor Variables: a Guide To Their Identification and Interpretation. Educational and Psychological Measurement 34: 35-46

Connor CG. 2003. Treatment of Dry Eye with a Transdermal 3% Testosterone Cream. Investigative Ophthalmology & Visual Science 44: E Abstract 2450

Cornell-Bell AH, Sullivan DA, Allansmith MR. 1985. Gender-related differences in the morphology of the lacrimal gland. Investigative Ophthalmology & Visual Science 26: 1170-5

Cousen P, Cackett P, Bennett H, Swa K, Dhillon B. 2007. Tear production and corneal sensitivity in diabetes. Journal of Diabetes and its Complications 21: 371-73

Courville T, Thompson B. 2001. Use of Structure Coefficients in Published Multiple Regression Articles: β is not Enough. Educational and Psychological Measurement 61: 229-48

Couzinet B, Meduri G, Lecce MG, Young J, Brailly S, et al. 2001. The postmenopausal ovary is not a major androgen-producing gland. Journal of Clinical Endocrinology & Metabolism 86: 5060-66

Craig JP, Willcox MDP, Argüeso P, Maissa C, Stahl U, et al. 2013. The TFOS International Workshop on Contact Lens Discomfort: Report of the Contact Lens Interactions With the Tear Film Subcommittee. Investigative Ophthalmology & Visual Science 54: TFOS123-TFOS56

Darwish T, Brahma A, O'Donnell C, Efron N. 2007 Subbasal nerve fiber regeneration after LASIK and LASEK assessed by noncontact esthesiometry and in vivo confocal microscopy: prospective study. Journal of Cataract & Refractive Surgery 33: 1515-21

Dastjerdi MH, Dana R. 2009. Corneal Nerve Alterations in Dry Eye-associated Ocular Surface Disease. International Ophthalmology Clinics 49: 11-20

Davison S, Bell R, Donath S, Montalto J, Davis S. 2005. Androgen levels in adult females: changes with age, menopause, and oophorectomy. Journal of Clinical Endocrinology & Metabolism 90: 3847-53

De Boever J, Kohen F, Usanachitt C, Vandekerckhove D, Leyseele D, Vandewalle L. 1986. Direct chemiluminescence immunoassay for estradiol in serum. Clinical Chemistry 32: 1895-900

De Jong-van den Berg LT, Faber A, van den Berg PB. 2006. HRT use in 2001 and 2004 in The Netherlands—a world of difference. Maturitas 54: 193-97

De Paiva C, Pflugfelder SC. 2004. Corneal epitheliopathy of dry eye induces hyperesthesia to mechanical air jet stimulation. American Journal of Ophthalmology 137: 109-15

Delaveris N, Stahl UG, Madigan M, Jalbert I. 2011. 13th Scientific Meeting in Optometry and the 7th Optometric Educators Meeting. Clinical Experimental Optometry 94: e1-11

Den S, Shimizu K, Ikeda T, Tsubota K, Shimmura S, Shimazaki J. 2006. Association Between Meibomian Gland Changes and Aging, Sex, or Tear Function. Cornea 25: 651-55

Ding J, Sullivan DA. 2012. Aging and dry eye disease. Experimental gerontology 47: 483-90

Doria A, Iaccarino L, Sarzi-Puttini P, Ghirardello A, Zampieri S, et al. 2006. Estrogens in pregnancy and systemic lupus erythematosus. Annals of the New York Academy of Sciences 1069: 247-56

Dougherty BE, Nichols JJ, Nichols KK. 2011. Rasch analysis of the ocular surface disease index (OSDI). Investigative Ophthalmology & Visual Science 52: 8630-35

Doughty M, Fonn D, Richter D, Simpson T, Caffery B, Gordon K. 1997. A patient questionnaire approach to estimating the prevalence of dry eye symptoms in patients presenting to optometric practices across Canada. Optometry &Vision Science 74: 624-31

Doughty MJ, Whyte J, Li W. 2007. The phenol red thread test for lacrimal volume--does it matter if the eyes are open or closed? Ophthalmic & Physiological Optics : 27: 482-9

Efron N, Young G, Brennan NA. 1989. Ocular surface temperature. Current Eye Research 8: 901-06

Elmlinger MW, Kühnel W, Ranke MB. 2002. Reference ranges for serum concentrations of lutropin (LH), follitropin (FSH), estradiol (E2), prolactin, progesterone, sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), cortisol and ferritin in neonates, children and young adults. Clinical Chemistry and Laboratory Medicine 40: 1151-60

Erdem U, Ozdegirmenci O, Sobaci E, Sobaci G, Göktolga U, Dagli S. 2007. Dry eye in post-menopausal women using hormone replacement therapy. Maturitas 56: 257-62

Evans KSE, North RV, Purslow C. 2009. Tear ferning in contact lens wearers. Ophthalmic and Physiological Optics 29: 199-204

Farris RL, Stuchell RN, Mandel ID. 1986. Tear osmolarity variation in the dry eye. Transactions of the American Ophthalmological Society 84: 250-68

Feenstra RPG, Tseng SCG. 1992. Comparison of fluorescein and rose bengal staining. Ophthalmology 99: 605-17

Feldman F, Bain J, Matuk AR. 1978. Daily assessment of ocular and hormonal variables throughout the menstrual cycle. Archives of Ophthalmology 96: 1835-38

Fillingim RB, Edwards RR. 2001. The association of hormone replacement therapy with experimental pain responses in postmenopausal women. Pain 92: 229-34

Fogle RH, Stanczyk FZ, Zhang X, Paulson RJ. 2007. Ovarian androgen production in postmenopausal women. Journal of Clinical Endocrinology & Metabolism 92: 3040-43

Forman LJ, Tingle V, Estilow S, Cater J. 1989. The response to analgesia testing is affected by gonadal steroids in the rat. Life Sciences 45: 447-54

Forsblad-d'Elia H, Carlsten H, Labrie F, Konttinen YT, Ohlsson C. 2009. Low Serum Levels of Sex Steroids Are Associated with Disease Characteristics in Primary Sjogren's Syndrome; Supplementation with Dehydroepiandrosterone Restores the Concentrations. Journal of Clinical Endocrinology &Metabolism 94: 2044-51

Foulks G, Bron A. 2003. Meibomian Gland Dysfunction: A Clinical Scheme for Description, Diagnosis, Classification and Grading. The Ocular Surface 1: 107-26

Frye CA, Seliga AM. 2001. Testosterone increases analgesia, anxiolysis, and cognitive performance of male rats. Cognitive, Affective and Behavioral Neuroscience 1: 371-81

Gagliano C, Caruso S, Napolitano G, Malaguarnera G, Cicinelli MV, et al. 2014. Low levels of 17-β-oestradiol, oestrone and testosterone correlate with severe evaporative dysfunctional tear syndrome in postmenopausal women: a case–control study. British Journal of Ophthalmology 98: 371-6

García-Pérez MA. 2001. Yes-No Staircases with Fixed Step Sizes: Psychometric Properties and Optimal Setup. Optometry & Vision Science 78: 56-64

Gauthaman K, Ganesan AP. 2008. The hormonal effects of Tribulus terrestris and its role in the management of male erectile dysfunction – an evaluation using primates, rabbit and rat. Phytomedicine 15: 44-54

Gold EB, Bromberger J, Crawford S, Samuels S, Greendale GA, et al. 2001. Factors Associated with Age at Natural Menopause in a Multiethnic Sample of Midlife Women. American Journal of Epidemiology 153: 865-74

Goldsmith SJ. 1975. Radioimmunoassay: Review of basic principles. Seminars in Nuclear Medicine 5: 125-52

Golebiowski B. 2005. The Sensitivity Of The Ocular Surface During Contact Lens Wear. PhD Thesis

Golebiowski B, Lim M, Papas E, Stapleton F. 2013. Understanding the stimulus of an air-jet aesthesiometer: computerised modelling and subjective interpretation. Ophthalmic and Physiological Optics 33: 104-13

Golebiowski B, Papas E, Stapleton F. 2005. Corneal mechanical sensitivity measurement using a staircase technique. Ophthalmic and Physiological Optics 25: 246-53

Golebiowski B, Papas E, Stapleton F. 2011. Assessing the sensory function of the ocular surface: Implications of use of a non-contact air jet aesthesiometer versus the Cochet-Bonnet aesthesiometer. Experimental Eye Research 92: 408-13

Golebiowski B, Papas EB, Stapleton F. 2008. Factors Affecting Corneal and Conjunctival Sensitivity Measurement. Optometry and Vision Science 85: 241-46

Gordon GB, Bush TL, Helzlsouer KJ, Miller SR, Comstock GW. 1990. Relationship of Serum Levels of Dehydroepiandrosterone and Dehydroepiandrosterone Sulfate to the Risk of Developing Postmenopausal Breast Cancer. Cancer Research 50: 3859-62

Gorodeski G. 1992. Menopause: symptoms, sequelae and the risks and benefits of hormone replacement therapy. Current Affective Illness 11: 5-14

Guaschino S, Grimaldi E, Sartore A, Mugittu R, Mangino F, et al. 2003. Visual function in menopause: the role of hormone replacement therapy. Menopause 10: 53-57

Guillemin I, Begley C, Chalmers R, Baudouin C, Arnould B. 2012. Appraisal of Patient-Reported Outcome Instruments Available for Randomized Clinical Trials in Dry Eye: Revisiting the Standards. The Ocular Surface 10: 84-99

Guillon M, Maïssa C. 2010. Tear film evaporation-Effect of age and gender. Contact Lens and Anterior Eye 33: 171-75

Gulati A, Sullivan R, Buring JE, Sullivan DA, Dana R, Schaumberg DA. 2006. Validation and Repeatability of a Short Questionnaire for Dry Eye Syndrome. American Journal of Ophthalmology 142: 125-31.

Gunnarsson D, Selstam G, Ridderstrale Y, Holm L, Ekstedt E, Madej A. 2009. Effects of dietary phytoestrogens on plasma testosterone and triiodothyronine (T3) levels in male goat kids. Acta Veterinaria Scandinavica 51: 51

Gupta PD, Johar Sr K, Nagpal K, Vasavada AR. 2005. Sex Hormone Receptors in the Human Eye. Survey of ophthalmology 50: 274-84

Hagele JE, Guzek JP, Shavlik GW. 1994. Lacrimal Testing: Age as a Factor in Jones Testing. Ophthalmology 101: 612-17

Haines CJ, Xing S-M, Park K-H, Holinka CF, Ausmanas MK. 2005. Prevalence of menopausal symptoms in different ethnic groups of Asian women and responsiveness to therapy with three doses of conjugated estrogens/medroxyprogesterone acetate: The Pan-Asia menopause (PAM) study. Maturitas 52: 264-76

Hall JM, Couse JF, Korach KS. 2001. The Multifaceted Mechanisms of Estradiol and Estrogen Receptor Signaling. Journal of Biological Chemistry 276: 36869-72

Han SB, Hyon JY, Wee WR, Lee JH, Lee YJ, Yun P-Y. 2010. Reduced corneal sensitivity in patients with primary Sjögren's syndrome. Acta Ophthalmologica 88: 277-78

Hankinson SE, Manson JE, Spiegelman D, Willett WC, Longcope C, Speizer FE. 1995. Reproducibility of plasma hormone levels in postmenopausal women over a 2-3-year period. Cancer Epidemiology Biomarkers & Prevention 4: 649-54

Hay EM, Thomas E, Pal B, Hajeer A, Chambers H, Silman AJ. 1998. Weak association between subjective symptoms of and objective testing for dry eyes and dry mouth: results from a population based study. Annals of the Rheumatic Diseases 57: 20-24

Herren RY. 1933. The effect of high and low female sex hormone concentration on the two-point threshold of pain and touch and upon tactile sensitivity. Journal of Experimental Psychology 16: 324-7

Hilditch JR, Lewis J, Peter A, van Maris B, Ross A, et al. 1996. A menopause-specific quality of life questionnaire: development and psychometric properties. Maturitas 24: 161-75

Hilditch JR, Chen S, Norton PG, Lewis J. 1999. Experience of menopausal symptoms by Chinese and Canadian women. Climacteric 2: 164-73

Ho CK, Stoddart M, Walton M, Anderson RA, Beckett GJ. 2006. Calculated free testosterone in men: comparison of four equations and with free androgen index. Annals of Clinical Biochemistry 43: 389-97

Hykin P, Bron A. 1992. Age-related morphological changes in lid margin and meibomian gland anatomy. Cornea 11: 334-42

Jalbert I, Chao C, Ashley B, Stapleton F, Golebiowski B. 2012. Assessment of corneal sensitivity, corneal sub-basal nerve morphology, tear concentration of Substance P and discomfort symptoms in contact lens wearers and non-wearers. Optometry & Vision Science 89: E-abstract 120722

Jalbert I, Sweeney DF, Holden BA. 1999. The characteristics of corneal staining in successful daily and extended disposable contact lens wearers. Clinical and Experimental Optometry 82: 4-10

Jayaraman A, Pike CJ. 2009. Progesterone Attenuates Oestrogen Neuroprotection Via Downregulation of Oestrogen Receptor Expressibility in Cultured Neurones. Journal of Neuroendocrinology 21: 77-81

Jensen MP, Karoly P. 1991. Motivation and expectancy factors in symptom perception: A laboratory study of the placebo effect. Psychosomatic Medicine 53: 144-52

Jie Y, Xu L, Wu YY, Jonas JB. 2009. Prevalence of dry eye among adult Chinese in the Beijing Eye Study. Eye & Contact Lens 23: 688-93

Johnson ME. 2009. The Association Between Symptoms of Discomfort and Signs in Dry Eye. The Ocular Surface 7: 199-211

Johnson ME, Murphy PJ. 2007. Measurement of Ocular Surface Irritation on a Linear Interval Scale with the Ocular Comfort Index. Investigative Ophthalmology & Visual Science 48: 4451-58

Jordan V, Collins MM, Rowsby L, Prestwich G. 1977. A monohydroxylated metabolite of tamoxifen with potent antioestrogenic activity. Journal of Endocrinology 75: 305-16

Julius D, Basbaum AI. 2001. Molecular mechanisms of nociception. Nature 413: 203-10

Jung HM, Jung JW, Shin KH, Paik HJ. 2010. Effect of Hormone Replacement Therapy on Dry Eye Syndrome in Postmenopausal Women: A Prospective Study. Journal of Korean Ophthalmological Society 51: 175-79

Kampa M, Pelekanou V, Castanas E. 2008. Membrane-initiated steroid action in breast and prostate cancer. Steroids 73: 953-60

Khandelwal P, Liu S, Sullivan DA. 2012. Androgen regulation of gene expressibility in human meibomian gland and conjunctival epithelial cells. Molecular vision 18: 1055

Kiely PM, Carney LG, Smith G. 1983. Menstrual cycle variations of corneal topography and thickness. American Journal of Optometry and Physiological Optics 60: 822-29

Kim KW, Han SB, Han ER, Woo SJ, Lee JJ, et al. 2011. Association between depression and dry eye disease in an elderly population. Investigative Ophthalmology & Visual Science 52: 7954-58

Koh S, Watanabe H, Hosohata J, et al. 2003. Diagnosing dry eye using a blue-free barrier filter. American Journal of Ophthalmology 136:513–9

Krenzer KL, Reza Dana M, Ullman MD, Cermak JM, Tolls DB, et al. 2000. Effect of Androgen Deficiency on the Human Meibomian Gland and Ocular Surface. Journal of Clinical Endocrinology and Metabolism 85: 4874-82

Kuba T, Wu H-BK, Nazarian A, Festa ED, Barr GA, et al. 2006. Estradiol and progesterone differentially regulate formalin-induced nociception in ovariectomized female rats. Hormones and Behavior 49: 441-49

Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, et al. 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138: 863-70

Kunz R, Vist G, Oxman A. 2007. Randomisation to protect against selection bias in healthcare trials. Cochrane Database of Systemic Reviews 2: (4) Mr000012

Kuscu NK, Toprak AB, Vatansever S, Koyuncu FM, Guler C. 2003. Tear function changes of postmenopausal women in response to hormone replacement therapy. Maturitas 44: 63-68

Labrie F. 1991. Intracrinology. Molecular and Cellular Endocrinology 78: C113-C18

Labrie F. 2010. DHEA, important source of sex steroids in men and even more in women. Progress in Brain Research 182: 94-148

Labrie F, Bélanger A, Bélanger P, Bérubé R, Martel C, et al. 2006. Androgen glucuronides, instead of testosterone, as the new markers of androgenic activity in women. The Journal of Steroid Biochemistry and Molecular Biology 99: 182-88

Labrie F, Bélanger A, Cusan L, Gomez J-L, Candas B. 1997. Marked Decline in Serum Concentrations of Adrenal C19 Sex Steroid Precursors and Conjugated Androgen Metabolites During Aging. Journal of Clinical Endocrinology & Metabolism 82: 2396-402

Labrie F, Bélanger A, Labrie C, Simard J, Cusan L, et al. 1998. DHEA and the intracrine formation of androgens and estrogens in peripheral target tissues: its role during aging. Steroids 63: 322-28

Labrie F, BÉLanger A, Simard J, Luu-The VAN, Labrie C. 1995. DHEA and Peripheral Androgen and Estrogen Formation: Intracrinology. Annals of the New York Academy of Sciences 774: 16-28

Labrie F, Luu-The V, Labrie C, Bélanger A, Simard J, et al. 2003. Endocrine and Intracrine Sources of Androgens in Women: Inhibition of Breast Cancer and Other Roles of Androgens and Their Precursor Dehydroepiandrosterone. Endocrine Reviews 24: 152-82

Lamberts DW, Foster CS, Perry HD. 1979. Schirmer Test After Topical Anesthesia and the Tear Meniscus Height in Normal Eyes. Archives of Ophthalmology 97: 1082-85

Lamberts SWJ, van den Beld AW, van der Lely A-J. 1997. The Endocrinology of Aging. Science 278: 419-24

Langer RD. 2009. Efficacy, Safety, and Tolerability of Low-Dose Hormone Therapy in Managing Menopausal Symptoms. The Journal of the American Board of Family Medicine 22: 563-73

Lawrenson JG, Ruskell GL. 1993. Investigation of limbal touch sensitivity using a Cochet-Bonnet aesthesiometer. British Journal of Ophthalmology 77: 339-43

Lee AJ, Lee J, Saw S-M, Gazzard G, Koh D, et al. 2002. Prevalence and risk factors associated with dry eye symptoms: a population based study in Indonesia. British Journal of Ophthalmology 86: 1347-51

Lekhanont K, Rojanaporn D, Chuck RS, Vongthongsri A. 2006. Prevalence of dry eye in Bangkok, Thailand. Cornea 25: 1162-7

Lemp M, Bron A, Baudouin C, itez Del Castillo J, Geffen D, et al. 2011. Tear osmolarity in the diagnosis and management of dry eye disease. American Journal of Ophthalmology 151: 792 - 98

Lemp MA, Baudouin C, Baum J, Dogru M, Foulks GN, et al. 2007. Report of the Dry Eye WorkShop:The Definition and Classification of Dry Eye Disease. The Ocular Surface 5: 65-204

Lephart ED. 1996. A review of brain aromatase cytochrome P450. Brain Research Reviews 22: 1-26

Ligniers BD, Basdevant A, Thomas G, Thalabard J-C, Mercier-Bodard C, et al. 1986. Biological Effects of Estradiol-17β in Postmenopausal Women: Oral Versus percutaneous Administration. Journal of Clinical Endocrinology & Metabolism 62: 536-41

Lin P-Y, Tsai S-Y, Cheng C-Y, Liu J-H, Chou P, Hsu W-M. 2003. Prevalence of dry eye among an elderly Chinese population in Taiwan: The Shihpai Eye Study. Ophthalmology 110: 1096-101

Liesegang TJ. 2008. Herpes zoster ophthalmicus: natural history, risk factors, clinical presentation, and morbidity. *Ophthalmology* 115: S3-S12

Lum E. 2013. The Impact of Overnight Orthokeratology on Sub-Basal Nerve Plexus Morphology and Corneal Sensitivity'. PhD Thesis

Luo L, Li D-Q, Doshi A, Farley W, Corrales RM, Pflugfelder SC. 2004. Experimental Dry Eye Stimulates Production of Inflammatory Cytokines and MMP-9 and Activates MAPK Signaling Pathways on the Ocular Surface. Investigative Ophthalmology & Visual Science 45: 4293-301

Luu-The V, Labrie F. 2010. Chapter 10 - The Intracrine Sex Steroid Biosynthesis Pathways In Progress in Brain Research, ed. M Luciano, pp. 177-92: Elsevier

Ma B, Yu L-h, Fan J, Cong B, He P, et al. 2011. Estrogen modulation of peripheral pain signal transduction: involvement of P2X3 receptors. Purinergic Signalling 7: 73-83

Ma H, Shieh K-J, Lee S-L. 2006. Study of ELISA Technique. National Science. 2006; 4: 36-7

Machin D, Campbell MJ, Tan S-B, Tan S-H. 2011. Sample size tables for clinical studies. John Wiley & Sons.

MacIver M, Tanelian D. 1993. Structural and functional specialization of A delta and C fiber free nerve endings innervating rabbit corneal epithelium. The Journal of Neuroscience 13: 4511-24

Macri A, Pflugfelder S. 2000. Correlation of the Schirmer 1 and Fluorescein Clearance Tests With the Severity of Corneal Epithelial and Eyelid Disease. Archives of Ophthalmology 118: 1632-38

Macri A, Rolando M, Pflugfelder S. 2000. A standardized visual scale for evaluation of tear fluorescein clearance. Ophthalmology 107: 1338-43

Mamalis N, Harrison D, Hiura G. 1996. Dry eyes and testosterone deficiency in women. Abstracts of the Centennial Annual Meeting of the American Academy of Ophthalmology, Chicago, IL. 132

Marcozzi G, Liberati V, Madia F, Pizzinga A, de Feo G. 2003. Effect of hormone replacement therapy on lacrimal fluid peroxidase activity in women. Maturitas 45: 225-29

Marks AR. 1997. Intracellular calcium-release channels: regulators of cell life and death. The American Journal of Physiology 272: H597-605

Mathers WD. 2000. Why the Eye Becomes Dry: A Cornea and Lacrimal Gland Feedback Model. Eye & Contact Lens 26: 159-65

Mathers WD, Dolney AM, Kraemer D. 2002. The Effect Of Hormone Replacement Therapy on the Symptoms and Physiologic Parameters of Dry Eye In Lacrimal Gland, Tear Film and Dry Eye Syndromes 3, ed. D Sullivan, pp. 1017-21: Kluwer Academic/Plenum Publishers

Mathers WD, Lane JA. 1998. Meibomian Gland Lipids, evaporation and tear film stability. Advances in Experimental Medicine and Biology 438: 349-60

Mathers WD, Lane JA, Zimmerman MB. 1996. Tear film changes associated with normal aging. Cornea 15: 229-34

Mathers WD, Shields WJ, Sachdev MS, Petroll WM, Jester JV. 1991. Meibomian Gland Dysfunction in Chronic Blepharitis. Cornea 10: 277-85

Mathers WD, Stovall D, Lane JABLS, Zimmerman MB, Johnson S. 1998. Menopause and Tear Function: The Influence of Prolactin and Sex Hormones on Human Tear Production. Cornea 17: 353-58

Mattke S, Balakrishnan A, Bergamo G, Newberry SJ. 2007. A review of methods to measure health-related productivy loss. American Journal of Managed Care 13: 211-17

McCarty C, Bansal A, Livingston P, Stanislavsky Y, Taylor H. 1998. The epidemiology of dry eye in Melbourne, Australia. Ophthalmology 105: 1114-19

McKown RL, Wang N, Raab RW, Karnati R, Zhang Y, et al. 2009. Lacritin and other new proteins of the lacrimal functional unit. Experimental Eye Research 88: 848-58

McShane LM, Dorgan JF, Greenhut S, Damato JJ. 1996. Reliability and validity of serum sex hormone measurements. Cancer Epidemiology Biomarkers & Prevention 5: 923-28

Mengher LS, Bron AJ, Tonge SR, Gilbert DJ. 1985. A non-invasive instrument for clinical assessment of the pre-corneal tear film stability. Current Eye Research 4: 1-7

Metka M EH, Knogler W, Schurz B, Aichmair H. 1991. Ophthalmic Complaints as a Climacteric Symptom. Maturitas 14: 3-8

Michels G, Hoppe UC. 2008. Rapid actions of androgens. Frontiers in Neuroendocrinology 29: 182-98

Miljanovic B, Dana R, Sullivan D, Schaumberg D. 2007. Impact of dry eye syndrome on vision-related quality of life. American Journal of Ophthalmology 143: 409 -15

Miller KK, Rosner W, Lee H, Hier J, Sesmilo G, et al. 2004. Measurement of Free Testosterone in Normal Women and Women with Androgen Deficiency: Comparison of Methods. Journal of Clinical Endocrinology & Metabolism 89: 525-33

Miller KL, Walt JG, Mink DR, Satram-Hoang S, Wilson SE, et al. 2010. Minimal Clinically Important Difference for the Ocular Surface Disease Index. Archives of Ophthalmology 128: 94-101

Millodot M. 1977a. The influence of age on the sensitivity of the cornea. Investigative Ophthalmology & Visual Science 16: 240-42

Millodot M. 1977b. The influence of pregnancy on the sensitivity of the cornea. British journal of ophthalmology 61: 646-9

Millodot M, Lamont A. 1974. Influence of menstruation on corneal sensitivity. British Journal of Ophthalmology 58: 752-56

Millodot ML, W. 1967. Effect of Bending of the Nylon Thread of the Cochet-Bonnet Corneal Aesthesiometer upon the Recorded Pressure. The Contact Lens 1: 5-6,28

Mocci F, Serra A, Corrias GA. 2001. Psychological factors and visual fatigue in working with video display terminals. Occupational and Environmental Medicine 58: 267-71

Moghassemi S, Ziaei S, Haidari Z. 2011. Female Sexual Dysfunction in Iranian Postmenopausal Women: Prevalence and Correlation with Hormonal Profile. Journal of Sexual Medicine 8: 3154-59

Morgan PB, Maldonado-Codina C. 2009. Corneal staining: Do we really understand what we are seeing? Contact Lens and Anterior Eye 32: 48-54

Morgan R. 2011. Hormone Replacement Therapy use in Australia has remained stable over the past two years (2010). Retrieved 9 July 2011 from http://www.roymorganonlinestore.com/News/1106-Hormone-Replacement-Therapy-use-in-Australi.aspx.

Moss S, Klein R, Klein B. 2008. Long-term incidence of dry eye in an older population. Optometry & Vision Science :85: 668-74

Moss SE, Klein R, Klein BEK. 2000. Prevalence of and Risk Factors for Dry Eye Syndrome. Archives of Ophthalmology 118: 1264-68

Murphy PJ, Lawrenson JG, Patel S, Marshall J. 1998 Reliability of the non-contact corneal aesthesiometer and its comparison with the Cochet-Bonnet aesthesiometer. Ophthalmic and Physiological Optics 18: 532-9

Murphy PJ, Patel S, Marshall J. 1996. A new non-contact corneal aesthesiometer (NCCA). Ophthalmic and Physiological Optics 16: 101-07

Murphy PJ, Patel S, Marshall J. 2001. The Effect of Long-term, Daily Contact Lens Wear on Corneal Sensitivity. Cornea 20: 264-69

Murphy TK, Calle EE, Rodriguez C, Kahn HS, Thun MJ. 2000. Body Mass Index and Colon Cancer Mortality in a Large Prospective Study. American Journal of Epidemiology 152: 847-54

Nagymihályi A, Dikstein S, Tiffany JM. 2004. The influence of eyelid temperature on the delivery of meibomian oil. Experimental Eye Research 78: 367-70

Nanavaty MA, Long M, Malhotra R. 2013. Transdermal androgen patches in evaporative dry eye syndrome with androgen deficiency: a pilot study. British Journal of Ophthalmology 98: 567-9

Nayebi AR, Ahmadiani A. 1999. Involvement of the spinal serotonergic system in analgesia produced by castration. Pharmacology Biochemistry and Behavior 64: 467-71

Nelson HD, Humphrey LL, Nygren P, Teutsch SM, Allan JD. 2002. Postmenopausal hormone replacement therapy: Scientific review. Journal of The American Medical Association 288: 872-81

Ness J, Aronow WS. 2006. Prevalence and causes of persistent use of hormone replacement therapy among postmenopausal women: a follow-up study. American Journal of Therapeutics 13: 109-12

Nichols JJ, Ziegler C, Mitchell GL, Nichols KK. 2005. Self-Reported Dry Eye Disease across Refractive Modalities. Investigative Ophthalmology & Visual Science 46: 1911-14

Nichols K, Jones L. 2008. Is Tear Osmolarity A Key Feature in Dry Eye in Post Menopausal Women? Investigative Ophthalmology & Visual Science 49: E Abstract 5284

Nichols K, Nichols J, Mitchell G. 2004. The lack of association between signs and symptoms in patients with dry eye disease. Cornea 23: 762-70

Nichols KK, Foulks GN, Bron AJ, Glasgow BJ, Dogru M, et al. 2011. The International Workshop on Meibomian Gland Dysfunction: Executive Summary. Investigative Ophthalmology & Visual Science 52: 1922-29

Nien C, Massei S, Lin G, et al. 2011. EFfects of age and dysfunction on human meibomian glands. Archives of Ophthalmology 129: 462-69

Norn M. 1987. Expressibility of Meibomian secretion. Acta Ophthalmologica 65: 137-42

Norn MS. 1969. Desiccation of the precorneal film. I. Corneal wetting time. Acta Ophthalmologica 47: 865-80

Norn MS. 1973. Conjunctival Sensitivity In Normal Eyes. Acta Ophthalmologica 51: 58-66

Örnek N, Dağ E, Örnek K. 2014. Corneal Sensitivity and Tear Function in Neurodegenerative Diseases. Current Eye Research June: 1-6

O'Brien PD, Collum LM. 2004. Dry eye: diagnosis and current treatment strategies. Current Allergy and Asthma Reports 4: 314-19

Olson MC, Korb DR, Greiner JV. 2003. Increase in Tear Film Lipid Layer Thickness Following Treatment with Warm Compresses in Patients with Meibomian Gland Dysfunction. Eye & Contact Lens 29: 96-99

Overlie I, Moen M, Morkrid L, Skjæraasen J, Holte A. 1999. The endocrine transition around menopause - five years prospective study with profiles of gonadotropines, estrogens, androgens and SHBG among healthy women. Acta Obstetricia et Gynecologica Scandinavica 78: 642-47

Ozcura F, Aydin S, Helvaci MR. 2007. Ocular surface disease index for the diagnosis of dry eye syndrome. Ocular Immunology and Inflammation 15: 389-93

Ozdemir M, Temizdemir H. 2009. Age- and gender-related tear function changes in normal population. Eye 24: 79-83

Panico S, Pisani P, Muti P, Recchione C, Cavalleri A, et al. 1990. Diurnal variation of testosterone and estradiol: a source of bias in comparative studies on breast cancer. Journal Of Endocrinological Investigation 13: 423-26

Panjari M, Davis SR. 2007. DHEA therapy for women: effect on sexual function and wellbeing. Human Reproduction Update 13: 239-48

Papas EB, Schultz BL. 1997. Repeatability and Comparison of Visual Analogue and Numerical Rating Scales in the Assessment of Visual Quality. Ophthalmic & Physiological Optics 6: 492-98

Paschides CA, Petroutsos G, Psilas K. 1991. Correlation of conjunctival impression cytology results with lacrimal function and age. Acta Ophthalmologica 69: 422-25

Patel S, Farrell JC. 1989. Age-related changes in precorneal tear film stability. Optometry & Vision Science : 66: 175-8

Patel VD, Watanabe JH, Strauss JA, Dubey AT. 2011. Work productivity loss in patients with dry eye disease: an online survey. Current Medical Research and Opinion 27: 1041-48

Pearce ST, Jordan VC. 2004. The biological role of estrogen receptors α and β in cancer. Critical Reviews in Oncology/Hematology 50: 3-22

Pflugfelder SC, Stern ME. 2004. Dry Eye and Ocular Surface Disorders Chapter 8 Sex and Sex Steroid Influences on Dry Eye Syndrome pp.165-75. Marcel Dekker Inc.

Pflugfelder SC, Tseng SCG, Sanabria O, Kell H, Garcia CG, et al. 1998. Evaluation of Subjective Assessments and Objective Diagnostic Tests for Diagnosing Tear-Film Disorders Known to Cause Ocular Irritation. Cornea 17: 38

Piwkumsribonruang N, Somboonporn W, Luanratanakorn P, Kaewrudee S, Tharnprisan P, Soontrapa S. 2010. Effectiveness of hormone therapy for treating dry eye syndrome in postmenopausal women: a randomized trial. Journal of the Medical Association of Thailand 93: 647-52

Porola P, Laine M, Virkki L, Poduval P, Konttinen YT. 2007. The Influence of Sex Steroids on Sjögren's Syndrome. Annals of the New York Academy of Sciences 1108: 426-32

Ray WA. 2003. Evaluating Medication Effects Outside of Clinical Trials: New-User Designs. American Journal of Epidemiology 158: 915-20

Riss B, Binder S, Riss P, Kemeter P. 1982. Corneal sensitivity during the menstrual cycle. British Journal of Ophthalmology 66: 123-26

Rocha EM, Wickham LA, da Silveira LA, Krenzer KL, Yu F-S, et al. 2000. Identification of androgen receptor protein and 5α-reductase mRNA in human ocular tissues. British Journal of Ophthalmology 84: 76-84

Rocha FJ, Alexandra Wickham L, Pena J, Gao J, Ono M, et al. 1993. Influence of gender and the endocrine environment on the distribution of androgen receptors in the lacrimal gland. The Journal of Steroid Biochemistry and Molecular Biology 46: 737-49

Rohr UD. 2002. The impact of testosterone imbalance on depression and women's health. Maturitas 41, Supplement 1: 25-46

Rolando M, Zierhut M. 2001. The Ocular Surface and Tear Film and Their Dysfunction in Dry Eye Disease. Survey of Ophthalmology 45: S203-S10

Romano GJ, Harlan RE, Shivers BD, Howells RD, Pfaff DW. 1988. Estrogen increases proenkephalin messenger ribonucleic acid levels in the ventromedial hypothalamus of the rat. Molecular Endocrinology 2: 1320-28

Rosenberg M. 1965. Society and the adolescent self-image. Princeton, NJ: Princeton University.

Rosner W, Auchus RJ, Azziz R, Sluss PM, Raff H. 2007. Utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement. Journal of Clinical Endocrinology & Metabolism 92: 405-13

Roszkowska AM, Colosi P, Ferreri FMB, Galasso S. 2004. Age-Related Modifications of Corneal Sensitivity. Ophthalmologica 218: 350-55

Ruskell G, L. 1985. Innervation of the conjunctiva. Transactions of The Ophthalmological Societies Of The United Kingdom 104: 390-95

Russo PA, Bouchard CS, Galasso JM. 2007. Extended-wear silicone hydrogel soft contact lenses in the management of moderate to severe dry eye signs and symptoms secondary to graft-versus-host disease. Eye and Contact Lens 33: 144-47

Sade de Paiva C, Lindsey JL, Pflugfelder SC. 2003. Assessing the severity of keratitis sicca with videokeratoscopic indices. Ophthalmology 110: 1102-09

Sade De Paiva C, Pflugfelder SC. 2004. Corneal epitheliopathy of dry eye induces hyperesthesia to mechanical air jet stimulation. American Journal of Ophthalmology 137: 109-15

Sahin OG, Kartal E. 2011. Meibomian Gland Dysfunction: Endocrine Aspects. Ophthalmology 2011: 6

Sakamoto R, Bennett ES, Henry VA, Paragina S, Narumi T, et al. 1993. The phenol red thread tear test: a cross-cultural study. Investigative Ophthalmology & Visual Science 34: 3510-4

Sator MO, Joura EA, Golaszewski T, Gruber D, Frigo P, et al. 1998. Treatment of menopausal keratoconjunctivitis sicca with topical oestradiol. BJOG: An International Journal of Obstetrics & Gynaecology 105: 100-02

Savini G, Prabhawasat P, Kojima T, Grueterich M, Espana E,Goto E. 2008. The challenge of dry eye diagnosis. Clinical ophthalmology *2*(1), 31.

Schäfer G, Krause W. 1985. The effect of estradiol on the sebaceous gland of the hamster ear and its antagonism by tamoxifen. Archives ofDermatology Research 277: 230-34

Schäfer M, Carter L, Stein C. 1994. Interleukin 1 beta and corticotropin-releasing factor inhibit pain by releasing opioids from immune cells in inflamed tissue. Proceedings of the National Academy of Sciences 91: 4219-23

Schaumberg D, Buring L, Sullivan D, Dana M. 2001. Hormone Replacement Therapy and Dry Eye Syndrome. Journal of The American Medical Association: 2114-19.

Schaumberg DA, Dana R, Buring JE, Sullivan DA. 2009. Prevalence of dry eye disease among us men: Estimates from the physicians& health studies. Archives of Ophthalmology 127: 763-68

Schaumberg DA, Nichols JJ, Papas EB, Tong L, Uchino M, Nichols KK. 2011. The International Workshop on Meibomian Gland Dysfunction: Report of the Subcommittee on the Epidemiology of, and Associated Risk Factors for, MGD. Investigative Ophthalmology & Visual Science 52: 1994-2005

Schaumberg DA, Sullivan DA, Buring JE, Dana MR. 2003. Prevalence of dry eye syndrome among US women. American Journal of Ophthalmology 136: 318-26

Schein O, Munoz B, Tielsch J, Bandeen-Roche K, West S. 1997a. Prevalence of dry eye among the elderly. American Journal of Ophthalmology 124: 723 - 28

Schein OD, Tielsch JM, Munoz B, Bandeen-Roche K, West S. 1997b. Relation between signs and symptoms of dry eye in the elderly. A population-based perspective. Ophthalmology 104: 1395-401

Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. 2000. Reliability and validity of the Ocular Surface Disease Index. Archives of Ophthalmology 118: 615-21

Schirra F, Suzuki T, Dickinson DP, Townsend DJ, Gipson IK, Sullivan DA. 2006. Identification of Steroidogenic Enzyme mRNAs in the Human Lacrimal Gland, Meibomian Gland, Cornea, and Conjunctiva. Cornea 25: 438-42

Schirra F, Suzuki T, Richards SM, Jensen RV, Liu M, et al. 2005. Androgen Control of Gene Expressibility in the Mouse Meibomian Gland. Investigative Ophthalmology & Visual Science 46: 3666-75

Scott G, Yiu SC, Wasilewski D, Song J, Smith RE. 2005. Combined esterified estrogen and methyltestosterone treatment for dry eye syndrome in postmenopausal women. American Journal of Ophthalmology 139: 1109-10

Scuderi G, Contestabile MT, Gagliano C, Iacovello D, Scuderi L, Avitabile T. 2012. Effects of phytoestrogen supplementation in postmenopausal women with dry eye syndrome: a randomized clinical trial. Canadian Journal of Ophthalmology 47: 489-92

Shibuya K, Takata N, Hojo Y, Furukawa A, Yasumatsu N, et al. 2003. Hippocampal cytochrome P450s synthesize brain neurosteroids which are paracrine neuromodulators of synaptic signal transduction. Biochimica et Biophysica Acta (BBA) - General Subjects 1619: 301-16

Shifren JL, Braunstein GD, Simon JA, Casson PR, Buster JE, et al. 2000. Transdermal Testosterone Treatment in Women with Impaired Sexual Function after Oophorectomy. New England Journal of Medicine 343: 682-88

Shimmura S, Shimazaki J, Tsubota K. 1999. Results of a Population-Based Questionnaire on the Symptoms and Lifestyles Associated with Dry Eye. Cornea 18: 408-11

Siak JJ, Tong L, Wong WL, Cajucom-Uy H, Rosman M, et al. 2012. Prevalence and risk factors of meibomian gland dysfunction: the Singapore Malay eye study. Cornea 31: 1223-8

Simental J, Sar M, Lane M, French F, Wilson E. 1991. Transcriptional activation and nuclear targeting signals of the human androgen receptor. Journal of Biological Chemistry 266: 510-18

Simmons P, Vehige J, Carlisle C, Felix C. 2003. Comparison of dry eye signs in self-described mild and moderate patients. Investigative Ophthalmology & Visual Science 44: 2448

Simpson TL, Situ P, Jones LW, Fonn D. 2008. Dry Eye Symptoms Assessed by Four Questionnaires. Optometry & Vision Science 85: 692-99

Sitruk-Ware RR. 2007. New hormonal therapies and regimens in the postmenopause: routes of administration and timing of initiation. Climacteric 10: 358-70

Situ P, Simpson TL, Fonn D, Jones LW. 2008a. Conjunctival and Corneal Pneumatic Sensitivity Is Associated with Signs and Symptoms of Ocular Dryness. Investigative Ophthalmology & Visual Science 49: 2971-76

Situ P, Simpson TL, Jones LW, Fonn D. 2008b. Conjunctival and Corneal Hyperesthesia in Subjects with Dryness Symptoms. Optometry & Vision Science 85: 867-72

Situ P, Simpson TL, Jones LW, Fonn D. 2010. Effects of Silicone Hydrogel Contact Lens Wear on Ocular Surface Sensitivity to Tactile, Pneumatic Mechanical, and Chemical Stimulation. Investigative Ophthalmology & Visual Science 51: 6111-17

Smeeth L, Iliffe S. 1998. Effectiveness of screening older people for impaired vision in community setting: systematic review of evidence from randomised controlled trials. British Medical Journal 316: 660-63

Smith JA, Albeitz J, Begley C, Caffery B, Nichols K, Schaumberg D. 2007. Report of the Epidemiology Subcommittee of the International Dry Eye Work Shop The Ocular Surface 5: 93-107

Smith JA, Vitale S, Reed GF, Grieshaber SA, Goodman LA, et al. 2004. Dry Eye Signs and Symptoms in Women With Premature Ovarian Failure. Archives of Ophthalmology 122: 151-56

Solomon JD, Blackie CA, Greiner J. 2008. Ophthalmology & Visual Science 49: E Abstract 5324.

Spoerl E, Zubaty V, Raiskup-Wolf F, Pillunat LE. 2007. Oestrogen-induced changes in biomechanics in the cornea as a possible reason for keratectasia. British Journal of Ophthalmology 91: 1547-50

Srinivasan S, Chan C, Jones L 2007. Apparent time-dependent differences in inferior tear meniscus height in human subjects with mild dry eye symptoms. Clinical and Experimental Optometry 90.5:345-350

Srinivasan S, Joyce E, Senchyna M, Simpson T, Jones L. 2008. Clinical signs and symptoms in post-menopausal females with symptoms of dry eye. Ophthalmic and Physiological Optics 28: 365-72

Stadberg E, Mattsson L-Å, Milsom IAN. 2000. Factors associated with climacteric symptoms and the use of hormone replacement therapy. Acta Obstetricia et Gynecologica Scandinavica 79: 286-92

Stanczyk FZ, Lee JS, Santen RJ. 2007. Standardization of Steroid Hormone Assays: Why, How, and When? Cancer Epidemiology Biomarkers & Prevention 16: 1713-19

Stapleton F, Marfurt C, Golebiowski B, Rosenblatt M, Bereiter D, et al. 2013. The TFOS International Workshop on Contact Lens Discomfort: Report of the Subcommittee on Neurobiology. Investigative Ophthalmology & Visual Science 54: TFOS71-TFOS97

Stapleton F, Tan ME, Papas EB, Ehrmann K, Golebiowski B, et al. 2004. Corneal and conjunctival sensitivity to air stimuli. British Journal of Ophthalmology 88: 1547-51

Steagall R, Yamagami H, Wickham L, Sullivan D. 2002. Androgen control of gene expressibility in the rabbit meibomian gland. Advances in Experimental Medicine And Biology 506: 465-76

Stern ME, Beuerman RW, Fox RI, Gao J, Mircheff AK, Pflugfelder SC. 1998. The pathology of dry eye: the interaction between the ocular surface and lacrimal glands. Cornea 17: 584-89

Stern ME, Gao J, Siemasko KF, Beuerman RW, Pflugfelder SC. 2004. The role of the lacrimal functional unit in the pathophysiology of dry eye. Experimental Eye Research 78: 409-16

Stevenson D, Tauber J, Reis BL. 2000. Efficacy and safety of cyclosporin A ophthalmic emulsion in the treatment of moderate-to-servere dry eye disease: A dose-ranging, randomized trial. Ophthalmology 107: 967-74

Stomati M, Hartman B, Spinetti A. 1996. Effects of hormonal replacement therapy on plasma sex hormone-binding globulin, androgen and insulin-like growth factor-1 levels in postmenopausal women. Journal of Endocrinology and Investigation 19: 535-41

Sturdee DW, Panay N. 2010. Recommendations for the management of postmenopausal vaginal atrophy. Climacteric 13: 509-22

Sullivan B, Evans J, Dana M, Sullivan D. 2002a. Impact of androgen deficiency on the lipid profiles in human meibomian gland secretions. Advances in Experimental Medicine and Biology 506: 449

Sullivan BD, Crews LA, Messmer EM, Foulks GN, Nichols KK, et al. 2014. Correlations between commonly used objective signs and symptoms for the diagnosis of dry eye disease: clinical implications. Acta Ophthalmologica 92: 161-6

Sullivan BD, Evans JE, Cermak JM, Krenzer KL, Dana MR, Sullivan DA. 2002. Complete Androgen Insensitivity Syndrome: Effect on Human Meibomian Gland Secretions. Archives of Ophthalmology 120: 1689-99

Sullivan D, Bloch K, Allansmith M. 1984. Hormonal influence on the secretory immune system of the eye: androgen control of secretory component production by the rat exorbital gland. Immunology 52: 239-46

Sullivan DA. 2004a. Sex and sex steroid influence on dry eye syndromes.Pflugfelder S, Beuerman R, Stern ME, editors. Dry Eye and Ocular Surface Diseasepp. 165-90. New York Marcel Dekker, Inc.

Sullivan DA. 2004b. Tearful relationships? Sex, hormones, the lacrimal gland, and aqueous-deficient dry eye. The Ocular Surface 2: 92-123

Sullivan DA, Bélanger A, Cermak JM, Bérubé R, Papas AS, et al. 2003. Are women with Sjögren's syndrome androgen-deficient? The Journal of Rheumatology 30: 2413-19

Sullivan DA, Block L, Pena JDO. 1996. Influence of androgens and pituitary hormones on the structural profile and secretory activity of the lacrimal gland. Acta Ophthalmologica Scandinavica 74: 421-35

Sullivan DA, Hann LE, Yee L, Allansmith MR. 1990. Age- and gender-related influence on the lacrimal gland and tears. Acta Ophthalmologica 68: 188-94

Sullivan DA, Jensen RV, Suzuki T, Richards SM. 2009. Do sex steroids exert sex-specific and/or opposite effects on gene expressibility in lacrimal and meibomian glands? Molecular Vision 15: 1553-72

Sullivan DA, Sullivan BD, Evans JE, Schirra F, Yamagami H, et al. 2002c. Androgen Deficiency, Meibomian Gland Dysfunction, and Evaporative Dry Eye. Annals of the New York Academy of Sciences 966: 211-22

Sullivan DA, Sullivan BD, Ullman MD, Rocha EM, Krenzer KL, et al. 2000. Androgen Influence on the Meibomian Gland. Investigative Ophthalmology & Visual Science 41: 3732-42

Suzuki T, Schirra F, Richards SM, Jensen RV, Sullivan DA. 2008. Estrogen and Progesterone Control of Gene Expressibility in the Mouse Meibomian Gland. Investigative Ophthalmology & Visual Science 49: 1797-808

Suzuki T, Sullivan DA. 2005. Estrogen stimulation of proinflammatory cytokine and matrix metalloproteinase gene expressibility in human corneal epithelial cells. Cornea 24: 1004-09

Swerdloff RS, Wang C. 1998. Dihydrotestosterone: a rationale for its use as a non-aromatizable androgen replacement therapeutic agent. Bailliere's Clinical Endocrinology And Metabolism 12: 501-6

Taiym S, Haghighat N, Al-Hashimi I. 2004. A comparison of the hormone levels in patients with Sjogren's syndrome and healthy controls. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology 97: 579-83

Takamatsu K, Ohta H, Kasuga M, Makita K, Horiguchi F, Nozawa S. 2001. Vaginal symptoms in Japanese postmenopausal women:comparison with other climacteric symptoms. Climacteric 4: 299-305

Taner P, Akarsu C, Atasoy P, Bayram M, Ergin A. 2004. The Effects of Hormone Replacement Therapy on Ocular Surface and Tear Function Tests in Postmenopausal Women. Ophthalmologica 218: 257-59

Thijssen JHH. 1988. Hormonal and Nonhormonal Factors Affecting Sex Hormone-Binding Globulin Levels in Blood. Annals of the New York Academy of Sciences 538: 280-86

Thody AJ, Shuster S. 1989. Control and function of sebaceous glands. Physiological Reviews 69: 383-416

Toker E, Asfuroglu E. 2010. Corneal and Conjunctival Sensitivity in Patients With Dry Eye: The Effect of Topical Cyclosporine Therapy. Cornea 29: 133-40

Tomlinson A, Bron AJ, Korb DR, Amano S, Paugh JR, et al. 2011. The International Workshop on Meibomian Gland Dysfunction: Report of the Diagnosis Subcommittee. Investigative Ophthalmology & Visual Science 52: 2006-49

Tomlinson A, Pearce EI, Simmons PA, Blades K. 2001. Effect of oral contraceptives on tear physiology. Ophthalmic and Physiological Optics 21: 9-16

Tomlinson A, Kenneth J, Blades K, Pearce EI. 2001. What does the phenol red thread test actually measure? Optometry & Vision Science 78.3 (2001): 142-146.

Tomlinson A, et al. 2006. Tear film osmolarity: determination of a referent for dry eye diagnosis. Investigative Ophthalmology & Visual Science 47.10 (2006): 4309-4315.

Tsai M-J, Clark JH, Schrader WT, O'Malley BW. 1998. Mechanisms of action of hormones that act as transcriptin-regulatory factors. Williams textbook of endocrinology. Philadelphia: WB Saunders.

Tuck MK, Chan DW, Chia D, Godwin AK, Grizzle WE, et al. 2008. Standard Operating Procedures for Serum and Plasma Collection: Early Detection Research Network Consensus Statement Standard Operating Procedure Integration Working Group. Journal of Proteome Research 8: 113-17

Tuisku IS, Konttinen YT, Konttinen LM, Tervo TM. 2008. Alterations in corneal sensitivity and nerve morphology in patients with primary Sjögren's syndrome. Experimental Eye Research 86: 879-85

Turner JA, Deyo RA, Loeser JD, Von Korff M, Fordyce WE. 1994. The importance of placebo effects in pain treatment and research.The Journal of The American Medical Association 271: 1609-14

Uchino M, Dogru M, Yagi Y, Goto E, Tomita M, et al. 2006. The features of dry eye disease in a Japanese elderly population. Optometry & Vision Science 83: 797-802

Uchino M, Schaumberg DA, Dogru M, Uchino Y, Fukagawa K, et al. 2008. Prevalence of dry eye disease among Japanese visual display terminal users. Ophthalmology 115: 1982-8

Uncu G, Avci R, Uncu Y, Kaymaz C, Develioğlu O. 2006. The effects of different hormone replacement therapy regimens on tear function, intraocular pressure and lens opacity. Gynecological Endocrinology 22: 501-05

Utian WH. 2004. Menopause-related definitions. International Congress Series 1266: 133-38

Vermeulen A, Giagulli VA. 1991. Physiopathology of plasma androstanediol-glucuronide. The Journal of Steroid Biochemistry and Molecular Biology 39: 829-33

Vermeulen A, Verdonck L, Kaufman JM. 1999. A critical evaluation of simple methods for the estimation of free testosterone in serum. Journal of Clinical Endocrinology &Metabolism 84: 3666-72

Versura P, Fresina M, Campos EC. 2007. Ocular surface changes over the menstrual cycle in women with and without dry eye. Gynecological Endocrinology 23: 385-90

Versura P, Frigato M, Cellini M, Mule R, Malavolta N, Campos EC. 2006. Diagnostic performance of tear function tests in Sjogren's syndrome patients. Eye 21: 229-37

Versura P, Profazio V, Campos EC. 2010. Performance of tear osmolarity compared to previous diagnostic tests for dry eye diseases. Current eye research 35.7 (2010): 553-564.

Viso E, Rodríguez-Ares MT, Abelenda D, Oubiña B, Gude F. 2012. Prevalence of Asymptomatic and Symptomatic Meibomian Gland Dysfunction in the General Population of Spain. Investigative Ophthalmology & Visual Science 53: 2601-06

Vitale S, Goodman LA, Reed GF, Smith JA. 2004. Health Quality Life Outcomes 2: 44

Vitali C, Bombardieri S, Jonsson R, Moutsopoulos H, Alexander E, et al. 2002. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Annals of the Rheumatic Diseases 61: 554 - 58

Wang C, Sh X, Chen X, Wu H, Zhang H, et al. 2012. 17-beta-estradiol inhibits hyperosmolarity-induced proinflammatory cytokine elevation via the p38 MAPK pathway in human corneal epithelial cells. Molecular Vision 18: 1115-22

Warne GL. 1997. Complete Androgen Insensitivity Syndrome. pp. 7, ed. RCsH Department of Endocrinology and Diabetes, Flemington Road, Parkville, Victoria 3052, Australia

Welt CK, Pagan YL, Smith PC, Rado KB, Hall JE. 2003. Control of Follicle-Stimulating Hormone by Estradiol and the Inhibins: Critical Role of Estradiol at the Hypothalamus during the Luteal-Follicular Transition. Journal of Clinical Endocrinology & Metabolism 88: 1766-71

Wickham LA, Gao J, Toda I, Rocha EM, Ono M, Sullivan DA. 2000. Identification of androgen, estrogen and progesterone receptor mRNAs in the eye. Acta Ophthalmologica Scandinavica 78: 146-53

Wilson G, Ren H, Laurent J. 1995. Corneal epithelial fluorescein staining. Journal of the American Optometric Association 66: 435-41

Women's Health Program Monash University. Testosterone and Androgen in Women (2010). Retrieved on 6th August 2011 from http://www.med.monash.edu.au

Wong. J, Ding. C, Yiu S, Smith R, Goodwin T, Schechter J. 2004. An Epidemiological study of pregnancy and dry eye. The Ocular Surface 3: S127

Xu K-P, Yagi Y, Tsubota K. 1996. Decrease in Corneal Sensitivity and Change in Tear Function in Dry Eye. Cornea 15: 235-39

Yamada H, Yokoi N, Komuro A, Maruyama K, Kinoshita S. 2005. Relationship Between Lid Margin Abnormalities and Meibography-Deteced Gland Dropout of Meibomian Gland. Investigative Ophthalmology &. Visual Science 46: E Abstract 4440

Yamaguchi M, Kutsuna M, Uno T, Zheng X, Kodama T, Ohashi Y. 2006. Marx Line: Fluorescein Staining Line on the Inner Lid as Indicator of Meibomian Gland Function. American Journal of Ophthalmology 141: 669-78

Yeh S, Pflugfelder S, Stern M. 2004. Pathological effects of lacrimal keratoconjunctivitis on the ocular surface Dry Eye and Ocular Surface Disorders pp.191-203New YorkMarcel Dekker

Yen SSC, Jaffe RB. 1991. Prolactin in human reproduction. Reproductive endocrinology 3rd Ed. pp. 357-388 Philadelphia WB Saunders.

Yokoi N, Kinoshita S. 1998. Importance of conjunctival epithelial evaluation in the diagnostic differentiation of dry eye from drug-induced epithelial keratopathy. Advances In Experimental Medicine And Biology438: 827-30

Yüksel B, Bozdağ B, Acar M, Topaloğlu E. 2010. Evaluation of the effect of topical cyclosporine A with impression cytology in dry eye patients. European Journal of Ophthalmology 20: 675-79

APPENDICES

Appendix 1

INFORMED CONSENT FOR STUDIES IN CHAPTER 2

THE UNIVERSITY OF NEW SOUTH WALES

PARTICIPANT INFORMATION STATEMENT AND CONSENT FORM

OCULAR SURFACE SENSITIVITY MEASUREMENTS USING THE BELMONTE OPM

You are invited to participate in a research study about the sensitivity of the frontal surface of the eye. We hope to compare sensitivity measured with two instruments developed just for this purpose. This is a pilot study conducted as part of a PhD research program. Your participation in this study is entirely voluntary. Please read the information below and ask questions about anything you do not understand, before deciding whether or not to participate.

- **PROCEDURES**

If you volunteer to participate in this study, you will be asked to do the following things:

Before the measurements are taken, a brief ocular history and a general eye's frontal surface (cornea) assessment will be carried out to ensure your suitability for the study

Part A

Sensitivity measurements will be taken from the central and slightly below of the right eye's frontal surface. You need to sit in front of an instrument (Belmonte OPM) and you will be presented with an air puff immediately after a blink. You will be asked to hold your eye open for the duration of puff and tell us whenever you could feel the puff. You will feel nothing or if you do, it will only be a mild sensation, similar to a gentle breeze and this procedure is not harmful to your eye. In the other instrument, a fine nylon thread will be used to gently touch your eye. Before beginning any experiment, we will demonstrate the techniques to you. The whole procedure shall take place about one hour and will be performed between12-3 pm.

Part B

Sensitivity measurements will be taken from the central and slightly below of the right eye's frontal surface. You need to sit in front of an instrument (Belmonte OPM) only and the above procedure will be repeated twice but shall be done with about an hour to a day apart.

You can choose to either participate in part A or B or both.

The whole procedure will be conducted at the UNSW School of Optometry and Vision Science building.

- **POTENTIAL RISKS AND DISCOMFORTS**

No significant damage has ever occurred in our hands from the usage of these instruments or the techniques mentioned above. Transient ocular discomfort, mild stinging or irritation, ocular fatigue and tearing are the only side effects which may occur within a few seconds. The probability that you will experience any of these side effects is minimal. Please inform the investigator should you experience these symptoms and examination by an optometrist would be arranged if necessary. We cannot and do not guarantee or promise that you will receive any benefits from this study.

- **CONFIDENTIALITY**

Any information obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission or as required by law. Findings will be reported as group results only and your individual identity will not be disclosed.

Any inquiries on the procedures can be directed to: *Ezai-0430211634 or email z3298344@student.unsw.edu.au*

Complaints may be directed to the Ethics Secretariat, The University of New South Wales, SYDNEY 2052 AUSTRALIA (phone 9385 4234, fax 9385 6648, email ethics.sec@unsw.edu.au). Any complaint you make will be investigated promptly and you will be informed out the outcome.

You will be given a copy of this form to keep**.**

INFORMED CONSENT FORM

THE UNIVERSITY OF NEW SOUTH WALES

OCULAR SURFACE SENSITIVITY MEASUREMENTS USING THE BELMONTE OPM

You are making a decision whether or not to participate. Your signature indicates that, having read the information provided above, you have decided to participate.

.....	.....
Signature of Research Participant	Signature of Witness
.....	.....
(Please PRINT name)	(Please PRINT name)
.....	.....
Date	Nature of Witness

REVOCATION OF CONSENT

I hereby wish to **WITHDRAW** my consent to participate in the research of **Ocular Surface Sensitivity Measurements Using The Belmonte OPM** proposal described above and understand that such withdrawal **WILL NOT** jeopardise any treatment or my relationship with The University of New South Wales, *(other participating organisation[s] or other professional[s]).*

.....	.....
Signature	Date
.....	
Please PRINT Name	

The section for Revocation of Consent should be forwarded to (Noor Ezailina Badarudin, School of Optometry and Vision Science, UNSW)

Appendix 2

INFORMED CONSENT FORM FOR STUDY IN CHAPTER 3

THE UNIVERSITY OF NEW SOUTH WALES

Dietary supplements and ocular comfort

PARTICIPANT INFORMATION STATEMENT

AND CONSENT FORM

SCHOOL OF OPTOMETRY AND VISION SCIENCE

Approval No 10110

Participant selection and purpose of study]
You *(i.e. the research participant)* are invited to participate in a study sponsored by Blackmores Ltd investigating the effects on the comfort of your eyes of supplementing your diet with nutritional oil capsules. These capsules contain omega oils and are a prototype formulation manufactured by Blackmores Ltd in Australia. These oil capsules are not currently marketed in Australia but The Therapeutics Goods Administration (TGA) has given approval for their limited use in this study. We *(i.e. the investigators)* hope to learn whether the oils contained in these capsules can reach the eyes' surface and improve the stability of your tears and optimise the health and comfort of your eyes.

You were selected as a participant in this study because you are 18 years of age or older, are in good health, have not been previously diagnosed with dry eye disease by a medical or ophthalmic practitioner, are not currently taking anticoagulant or blood thinning medication such as heparin, Warfarin, or aspirin and are not pregnant or breastfeeding. Both soft contact lens wearers and non-contact lens wearers are eligible to participate in this study. You should let us know whether you regularly wear contact lenses and if you change your contact lens wearing habits during the 3 month study period. It is important to advise us if, during the study period, your eating habits change significantly (e.g. if you start a diet or exercise program), if you become pregnant, if you are diagnosed with a new illness or if you begin to take any prescription or non-prescription medication.

[Description of study and risks]

If you decide to participate, you will be examined by us on 5 occasions: at baseline 1, baseline 2, at 1 month and after 3 months at final 1 and final 2. The interval between visits baseline 1 and baseline 2, and between final visit 1 and 2 will be no more than 3 days. At your baseline 2 visit, you will be given a supply of capsules to be taken by mouth. You will be asked to swallow 3 capsules with food once a day for 3 months. You will be one of 80 participants, 40 of whom will be given the prototype oil capsules and 40 of whom will be given "placebo" capsules containing no active ingredients. The type

of capsules (test or placebo) you receive will be randomly determined (like flipping a coin) and you will have equal chances of receiving one or the other. Neither we (the investigators) nor you (the research participant) will know which capsules you are taking (test or placebo) until the study is completed and the results are analysed.

In addition to regular optometric examination of the front surface of your eye, the following measurements will be taken at some of these visits.

- Questionnaires: You will be asked to fill in questionnaires about your eye comfort at some visits. At the baseline 1 visit you will also be asked to complete a short questionnaire about your nutrition.
- Ocular Sensitivity: The sensitivity of your eyes will be measured with two instruments. The first instrument will gently blow a puff of air towards the front surface of your eye and the second will use a fine nylon thread to gently touch your eye. The airflow or thread length at which you become aware of a sensation will be recorded. You may feel nothing or a slight awareness of the air or thread on the front surface of your eye.
- Tear Film Osmolality: The salt concentration of your tears will be measured. For this, a very small amount of tears (less than a tear drop) will be collected from just above your lower eyelid using a sterile device. The device will not touch your eye.
- Phenol Red Thread test: The ability of your eye to produce tears will be estimated by inserting a small piece of thread at your lower eyelid margin. You will be asked to keep your eyes open and blink normally for 15 seconds. You may feel a light foreign body sensation when the thread is inserted in the eye.
- Tear collection: A small amount of tears (less than a tear drop) will be collected from the lower eyelid using a small tube. This tube will not touch the cornea, but you may be aware of the tube touching your lower eyelid.
- Blood test: A small sample of blood will be collected by a professional pathology service located at UNSW campus to examine the levels of oils and hormones in your blood.
- Eyelid gland expressibility: We will gently massage your lower and upper eyelids to express the oil (meibum) naturally secreted by the eyelids. A small amount (less than a teaspoon) will be collected using a spatula or a clean filter paper. You may feel temporary awareness, slight discomfort or foreign body sensation as a result of this procedure.
- Impression cytology: Some surface cells from your eyes will be collected. Each eye will first be anesthetised using an eye drop. You may feel a slight stinging when the anaesthetic drop is instilled. A small sterile filter paper the size of your fingertip with be gently applied to the white part of your eye for a few seconds. Once the anaesthetic wears off, you may feel temporary slight discomfort or foreign body sensation as a result of this procedure. You should avoid rubbing your eyes for at least 30 minutes after this procedure and should advise us if you are aware that you have any allergies to drugs including anaesthetics and eye drops or their components.
- Confocal microscopy: Highly magnified images of the corneal nerves will be captured with a microscope. A lubricating tear gel will be placed in your eye to enable the microscope probe to come into contact with your eye and a drop of anaesthetic may be placed in your eye if

required. You should avoid rubbing your eyes for at least 30 minutes after this procedure and should advise us if you are aware that you have any allergies to drugs including anaesthetics and eye drops or their components.

- Photography / video: High resolution photographs and/or video recording of your eyes and eyelid margins may be taken. Photos and videos will be labelled with a unique subject number accessible only to personnel involved in the study. Confidentiality will be maintained at all times. Photographs and video recordings of your eyes may also be used for educational purposes.

We estimate that the baseline 1, 2 and final 1 and 2 visits will last approximately 1 hours and the 1-month visit approximately 30 minutes. We are not aware of any published or anecdotal reports listing damage from the procedures listed above. However, minor side effects such as burning, itching, irritation, excessive watering (tearing) of the eyes, sensitivity to light, andforeign body sensation may occur. These are temporary and will resolve quickly within hours. Dietary supplementation with the products in this study may rarely cause gastrointestinal irritation such as diarrhoea, heartburn, bloating or nausea, fishy body odour, fishy breath, blood thinning and bleeding. These effects are typically mild and temporary and reversible over time.

If any problems develop during the study, even if you do not think they are related to the study products, you should contact Drs Isabelle Jalbert or Blanka Golebiowski on (02) 93857623 (this number will forward to a mobile phone after hours). If you are unable to contact us, you should consult a doctor or go to the Emergency Department of your local hospital and advise them of your participation in this study.

Some dietary supplements have been shown to benefit health and can reduce pain, stiffness, fatigue, joint tenderness, and eye discomfort. We cannot and do not guarantee or promise that you will receive any such benefits from this study. Proven alternative treatments for eye discomfort and dryness exist including the use of artificial tear drops, anti-inflammatory eye drops, systemic drugs, environmental modifications such as humidifiers, and specialised eye devices such as punctal plugs. Your optometrist can provide more information on these alternative options and refer you to an appropriate practitioner should you desire it.

PARTICIPANT INFORMATION STATEMENT AND CONSENT FORM (continued)

Dietary supplements and ocular comfort

[Confidentiality and disclosure of information]
Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission, except as required by law. If you give us your permission by signing this document, we plan to publish the results in the scientific literature. In any publication, information will be provided in such a way that you cannot be identified.

[Recompense to participants]
At the end of the study, you will be provided with a year's complimentary supply of Blackmores' fish oil capsules and up to $50 in gift vouchers in lieu of reimbursement of expenses for the cost of travel to the UNSW.

Complaints may be directed to the Ethics Secretariat, The University of New South Wales, SYDNEY 2052 AUSTRALIA (phone 9385 4234, fax 9385 6648, email ethics.sec@unsw.edu.au). Any complaint you make will be investigated promptly and you will be informed out the outcome.

[Feedback to participants]
Should you indicate that you wish for us to do so by providing your email address below, we will email you with a summary of research findings on completion of this study. Your email address (optional):

_____

[Your consent]
Your decision whether or not to participate will not prejudice your future relations with the University of New South Wales and Blackmores Ltd. If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time without prejudice.

If you have any questions, please feel free to ask us. If you have any additional questions later, contact Dr Isabelle Jalbert on (02) 9385-9816 and she will be happy to answer them.

You will be given a copy of this form to keep.

THE UNIVERSITY OF NEW SOUTH WALES

PARTICIPANT INFORMATION STATEMENT AND CONSENT FORM (continued)

Dietary supplements and ocular comfort

You are making a decision whether or not to participate. Your signature indicates that, having read the information provided above, you have decided to participate.

.....	.....
Signature of Research	Participant Signature of Witness
.....	.....
(Please PRINT name)	(Please PRINT name) \
.....	.....
Date	**Nature of Witness**

Appendix 3

INFORMED CONSENT FORM FOR STUDIES IN CHAPTERS 4 & 5

Approval No #HC12087

THE UNIVERSITY OF NEW SOUTH WALES

SCHOOL OF OPTOMETRY AND VISION SCIENCE

PILOT STUDY ON THE EFFECTS OF HORMONE THERAPYON CLINICAL INDICATORS AND BIOMARKERS OF DRY EYE IN POST-MENOPAUSAL WOMEN

PARTICIPANT INFORMATION STATEMENT AND CONSENT FORM

THE UNIVERSITY OF NEW SOUTH WALES

You *(i.e. the research participant)* are invited to participate in a study investigating the effects of hormone treatment on the comfort of your eyes. We *(i.e. the investigators)*hope to learn whether the hormones contained in the treatment can improve the function of your tears to optimise the health and comfort of your eyes.

You were selected as a participant in this study because you are 50 years of age or older, have gone through menopause and in good health, have not been previously diagnosed with Sjögren's disease by a medical or ophthalmic practitioner and have not undergone hormone replacement therapy in the past 12 months. It is important to advise us, during the 8 week period, if you are diagnosed with a new illness, if you change any medications or if you begin to take any prescription or non-prescription medication, including eye drops or if you change your contact lens wearing habits.

If you decide to participate, you will be examined by us on 2 occasions: at baseline, and at 8 weeks. At your initial (baseline) visit, you will be given a supply of both gel and cream.The gel should be applied to a clean dry area of the skin on your inner thigh. The application surface area should be one to two times the size of your hand. You will be required to apply 0.5mL of the cream dailyontothe

inner thigh of the other leg and massage until vanished. You will be one of 40 participants, 10 of whom will receive testosterone therapy, 10 who will receive oestradiol therapy, 10 who will receive combination therapy and 10 who will receive a placebo (dummy) treatment. You will be allocated to one of these treatment groups and will have equal chances of being placed in one of the four groups. The type of treatment (active or placebo) you receive will be randomly determined and neither yourselfnor the examiners will know which group you have been assigned until the study is completed.

In addition to regular optometric examination of the front surface of your eye, the following measurements will be taken at each visit.

- Questionnaires: You will be asked to fill in questionnaires about your eye comfort at each visit. At baseline visit you will be asked 5 questionnaires to assess your general health and to understand more about your eyes and any symptoms of dry eye you may be experiencing and at the 8 week visit you will be asked to repeat 4 of these questionnaires to see if there have been any changes.
- Ocular Sensitivity: The sensitivity of your eyes will be measured with an instrument that gently touches a very thin nylon thread onto the front surface of your eye. The thickness of the thread at which you become aware of the sensation will be recorded. You may feel nothing or a slight awareness of the thread on the front surface of your eye.
- Tear Film Osmolarity: The salt concentration of your tears will be measured. For this, a very small amount of tears (less than a tear drop), will be collected from just above your lower eyelid using a sterile device. The device will not touch your eye.
- Phenol Red Thread test: The ability of your eye to produce tears will be estimated by inserting a small piece of thread into your lower eyelid margin. You will be asked to keep your eyes open and blink normally for 15 seconds. You may feel a light foreign body sensation when the thread is inserted in the eye.
- Schirmer Test: Your tear production will also be measured by placing a small filter paper strip into your lower eyelid margin. You will be asked to close your eyes for 5 minutes. You may feel a light foreign body sensation while the strip is in your eye.

PARTICIPANT INFORMATION STATEMENT AND CONSENT FORM (continued)

PILOT STUDY ON THE EFFECTS OF HORMONE THERAPY ON CLINICAL INDICATORS AND BIOMARKERS OF DRY EYE IN POST-MENOPAUSAL WOMEN

- Tear Film Break Up Time: The rate of which your tears evaporate from the front surface of your eye will be measured. The instrument that examines this will shine a bright light into your eye. Your eye will not be touched.
- Tear Collection: A small amount of tears (less than a tear drop) will be collected from the lower eyelid using a small tube. This tube will not touch the cornea, but you may be aware of the tube touching your lower eyelid.
- Venous Blood Collection: A small sample of blood (approximately a teaspoonful) will be collected by a professional pathology service located at the UNSW campus to examine the levels of hormones in your blood. As with any blood test, you may experience minor bruising or swelling at the side and/or light-headedness during the procedure. After the sample is taken, you are advised to avoid heavy lifting, and strenuous activities.
- Photography / video: High resolution photographs and/or video recording of your eyes and eyelid margins may be taken. Photos and videos will be labelled with a unique subject number accessible only to personnel involved in the study. Confidentiality will be maintained at all times. Photographs and video recordings of your eyes may also be used for educational purposes and in this case will be provided in such a way that you cannot be identified.

We estimate that the baseline and 8 week visits will last approximately 1 hour. We are not aware of any published or anecdotal reports listing damage from the procedures listed above. However, minor side effects such as burning, itching, irritation, excessive watering (tearing) of the eyes, sensitivity to light and foreign body sensation may occur. These are temporary and will resolve quickly within minutes to hours. The most common (in 1/10 cases) side effects of oestrogen include mild breast tenderness, vaginum spotting and application site reaction including possible itching and/or rash. Testosterone may cause mild acne in 1/10 cases. Other rare (1/100) long term side effects of testosterone and oestrogen therapies include:, headaches, dizziness, nausea, vomiting, fatigue, abdominal pain & distension, hirsutism (development of increased hair growth), depression, nervousness, abnormal bleeding from the uterus, cervical discharge, breast enlargement, weight changes, oedema, jaundice, swelling of the ankles, signs of virilisation (male physical characteristics), deepening of the voice, electrolyte disturbances &polycythemia (abnormally increased haemoglobin concentration in the blood), irregular heartbeat, varicose (dilated) veins,

leucorrhoea (white discharge from vagina). The side effects over the period of this trial are expected to be unlikely to occur. **These side effects have been reported with long term treatment only and all side effects are expected to resolve upon ceasing the product.**

If any problems develop during the study, even if you do not think they are related to the study products, you should contact Dr Blanka Golebiowski on (02) 9385 7623. If you are unable to contact us, you should consult a doctor or go to the Emergency Department of your local hospital and advise them of your participation in this study.

Some hormone supplements have been shown to benefit health and can reduce the symptoms and signs of dry eye disease. We cannot and do not guarantee or promise that you will receive any such benefits from this study. Proven alternative treatments for eye discomfort and dryness exist including the use of artificial tear drops, anti-inflammatory eye drops, systemic drugs, environmental modifications such as humidifiers, and specialised eye devices such as punctal plugs. Your optometrist can provide more information on these alternative options and refer you to an appropriate practitioner should you desire it.

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission, except as required by law. If you give us your permission by signing this document, we plan to publish the results in the scientific literature. In any publication, information will be provided in such a way that you cannot be identified.

At the end of the study, you will receive a $30 voucher per visit in lieu of reimbursement of expenses for the cost of travel to the UNSW.

THE UNIVERSITY OF NEW SOUTH WALES

PARTICIPANT INFORMATION STATEMENT AND CONSENT FORM (continued)

Pilot study on the Effects of Hormone Therapy on Clinical Indicators and Biomarkers of Dry Eye in Post-menopausal Women

Complaints may be directed to the Ethics Secretariat, The University of New South Wales, SYDNEY 2052 AUSTRALIA (phone 9385 4234, fax 9385 6648, email ethics.sec@unsw.edu.au). Any complaint you make will be investigated promptly and you will be informed out the outcome.

Should you indicate that you wish for us to do so by providing your email address below, we will email you with a summary of research findings on completion of this study. Your email address (optional):

_____

Your decision whether or not to participate will not prejudice your future relations with the University of New South Wales. If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time without prejudice.

If you have any questions, please feel free to ask us. If you have any additional questions later, contact Dr Blanka Golebiowski on (02) 9385 7623 and she will be happy to answer them.

You will be given a copy of this form to keep.

You are making a decision whether or not to participate. Your signature indicates that, having read the information provided above, you have decided to participate.

.....	.....
Signature of Research Participant	Signature of Witness
.....	.....
(Please PRINT name)	(Please PRINT name)
.....	.....
Date	Nature of Witness

THE UNIVERSITY OF NEW SOUTH WALES

PARTICIPANT INFORMATION STATEMENT AND CONSENT FORM (continued)

PILOT STUDY ON THE EFFECTS OF HORMONE THERAPY ON CLINICAL INDICATORS AND BIOMARKERS OF DRY EYE IN POST-MENOPAUSAL WOMEN

REVOCATION OF CONSENT

I hereby wish to **WITHDRAW** my consent to participate in the research proposal described above and understand that such withdrawal **WILL NOT** jeopardise any treatment or my relationship with The University of New South Wales.

....

Signature Date

.....

Please PRINT Name

The section for Revocation of Consent should be forwarded to Dr Blanka Golebiowski, School of Optometry and Vision Science, The University of New South Wales, Sydney 2052.

APPENDIX A WOMEN'S HEALTH STUDY (WHS) QUESTIONNAIRE

QUESTIONS	RESPONSES AVAILABLE			
HOW OFTEN DO YOUR EYES FELL DRY (NOT WET ENOUGH)?	NEVER	SOMETIMES	OFTEN	CONSTANTLY
HOW OFTEN DO YOUR EYES FELL IRRITATED?	NEVER	SOMETIMES	OFTEN	CONSTANTLY
HAVE YOU EVER BEEN DIAGNOSED (BY A CLINICIAN) AS HAVING DRY EYE SYNDROME?	YES		NO	

APPENDIX B OCULAR COMFORT INDEX

Ocular Comfort Index

ID:

This questionnaire was designed to grade the comfort of your eyes.
For each question please circle your answer.

Example: In the last week, how often were your eyes *red* ?

Never						Always
0	1	2	(3)	4	5	6

There are no right or wrong answers. Do not spend too long on any one question.

1 In the last week, how often did your eyes feel *dry* ?

Never						Always
0	1	2	3	4	5	6

When your eyes felt dry, typically, how intense was the *dryness* ?

Never had it						Severe
0	1	2	3	4	5	6

2 In the last week, how often did your eyes feel *gritty* ?

Never						Always
0	1	2	3	4	5	6

When your eyes felt gritty, typically, how intense was the *grittiness* ?

Never had it						Severe
0	1	2	3	4	5	6

3 In the last week, how often did your eyes feel *stingy* ?

Never						Always
0	1	2	3	4	5	6

When your eyes stung, typically, how intense was the *stinging* ?

Never had it						Severe
0	1	2	3	4	5	6

4 In the last week, how often did your eyes feel *tired* ?

Never						Always
0	1	2	3	4	5	6

When your eyes felt tired, typically, how intense was the *tiredness* ?

Never had it						Severe
0	1	2	3	4	5	6

5 In the last week, how often did your eyes feel *painful* ?

Never						Always
0	1	2	3	4	5	6

When your eyes felt painful, typically, how intense was the *pain* ?

Never had it						Severe
0	1	2	3	4	5	6

6 In the last week, how often did your eyes *itch* ?

Never						Always
0	1	2	3	4	5	6

When your eyes itched, typically, how intense was the *itching* ?

Never had it						Severe
0	1	2	3	4	5	6

APPENDIX C OSDI

Ocular Surface Disease Index© (OSDI©)[2]

Ask your patient the following 12 questions, and circle the number in the box that best represents each answer. Then, fill in boxes A, B, C, D, and E according to the instructions beside each.

HAVE YOU EXPERIENCED ANY OF THE FOLLOWING *DURING THE LAST WEEK*:

	All of the time	Most of the time	Half of the time	Some of the time	None of the time
1. Eyes that are sensitive to light?	4	3	2	1	0
2. Eyes that feel gritty?	4	3	2	1	0
3. Painful or sore eyes?	4	3	2	1	0
4. Blurred vision?	4	3	2	1	0
5. Poor vision?	4	3	2	1	0

Subtotal score for answers 1 to 5 (A)

HAVE PROBLEMS WITH YOUR EYES LIMITED YOU IN PERFORMING ANY OF THE FOLLOWING *DURING THE LAST WEEK*:

	All of the time	Most of the time	Half of the time	Some of the time	None of the time	
6. Reading?	4	3	2	1	0	N/A
7. Driving at night?	4	3	2	1	0	N/A
8. Working with a computer or bank machine (ATM)?	4	3	2	1	0	N/A
9. Watching TV?	4	3	2	1	0	N/A

Subtotal score for answers 6 to 9 (B)

HAVE YOUR EYES FELT UNCOMFORTABLE IN ANY OF THE FOLLOWING SITUATIONS *DURING THE LAST WEEK*:

	All of the time	Most of the time	Half of the time	Some of the time	None of the time	
10. Windy conditions?	4	3	2	1	0	N/A
11. Places or areas with low humidity (very dry)?	4	3	2	1	0	N/A
12. Areas that are air conditioned?	4	3	2	1	0	N/A

Subtotal score for answers 10 to 12 (C)

ADD SUBTOTALS A, B, AND C TO OBTAIN D (D = SUM OF SCORES FOR ALL QUESTIONS ANSWERED) (D)

TOTAL NUMBER OF QUESTIONS ANSWERED (DO NOT INCLUDE QUESTIONS ANSWERED N/A) (E)

Please turn over the questionnaire to calculate the patient's final OSDI© score.

APPENDIX D DRY EYE QUESTIONNAIRE (DEQ)

Patient Record	
Number:	_____
Date	_____
Time	_____

DRY EYE QUESTIONNAIRE

Please fill in the blank or circle the answer that best describes you. Choose only one answer per question.

1. What is your age?

   _____

2. What is your gender?
   1 Male
   2 Female

3. Have you worn contact lenses in the past?
   1 Yes
   2 No

4. If you have worn contact lenses in the past, which of the following did you wear most recently?

	Yes	No	Not Applicable
a. Rigid gas permeable	1	2	0
b. Disposable (lenses replaced frequently)	1	2	0
c. Soft daily wear (lenses replaced after 1 year or longer)	1	2	0
d. Extended wear (lenses worn overnight)	1	2	0

5. If you have worn contact lenses in the past, how important was each of the following issues in your decision to stop wearing contact lenses?

	Not at All Important				Very Important	Not Applicable
a. I never got used to the lenses	1	2	3	4	5	0
b. The lenses were uncomfortable all day	1	2	3	4	5	0
c. The lenses were most uncomfortable when first put in	1	2	3	4	5	0
d. The lenses became more uncomfortable later in the day	1	2	3	4	5	0
e. My eyes felt dry	1	2	3	4	5	0
f. The lenses felt scratchy and irritating	1	2	3	4	5	0
g. My vision was not clear enough	1	2	3	4	5	0
h. Wearing contact lenses was too much trouble	1	2	3	4	5	0
i. Other reason (please specify below)	1	2	3	4	5	0

_____

6. Questions about **EYE DISCOMFORT**:

a. During a typical day in the past week, **how often** did your eyes feel discomfort?

0 Never
1 Rarely
2 Sometimes
3 Frequently
4 Constantly

When your eyes felt discomfort, **how intense was this feeling of discomfort**…

b. Within the first two hours of getting up in the morning?

Never have it	Not at All Intense				Very Intense
0	**1**	**2**	**3**	**4**	**5**

c. At the end of the day, within two hours of going to bed?

Never have it	Not at All Intense				Very Intense
0	**1**	**2**	**3**	**4**	**5**

d. When your eyes felt discomfort, **how much did the discomfort bother you**?

Never have it	Not at All bothered				Extremely bothered
0	**1**	**2**	**3**	**4**	**5**

7. Questions about **EYE DRYNESS**:

a. During a typical day in the past week, **how often** did your eyes feel dry?

0 Never
1 Rarely
2 Sometimes
3 Frequently
4 Constantly

When your eyes felt dry, **how intense was this feeling of dryness**…

b. Within the first two hours of getting up in the morning?

Never have it	Not at All Intense				Very Intense
0	**1**	**2**	**3**	**4**	**5**

c. At the end of the day, within two hours of going to bed?

Never have it	Not at All Intense				Very Intense
0	**1**	**2**	**3**	**4**	**5**

d. When your eyes felt dry, **how much did the dryness bother you**?

Never have it	Not at All bothered				Extremely bothered
0	**1**	**2**	**3**	**4**	**5**

8. Questions about **EYE GRITTINESS AND SCRATCHINESS**:

a. During a typical day in the past week, **how often** did your eyes feel gritty and scratchy?

0 Never
1 Rarely
2 Sometimes
3 Frequently
4 Constantly

When your eyes felt grittiness and scratchiness, **how intense was this feeling of grittiness and scratchiness**…

b. Within the first two hours of getting up in the morning?

Never have it	Not at All Intense				Very Intense
0	**1**	**2**	**3**	**4**	**5**

c. At the end of the day, within two hours of going to bed?

Never have it	Not at All Intense				Very Intense
0	**1**	**2**	**3**	**4**	**5**

d. When your eyes felt gritty and scratchy, **how much did the grittiness and scratchiness bother you?**

Never have it	Not at All bothered				Extremely bothered
0	**1**	**2**	**3**	**4**	**5**

9. Questions about **EYE BURNING AND STINGING**:

a. During a typical day in the past week, **how often** did your eyes feel burning and stinging?

0 Never
1 Rarely
2 Sometimes
3 Frequently
4 Constantly

When your eyes felt burning and stinging, **how intense was this feeling burning and stinging** …

b. Within the first two hours of getting up in the morning?

Never have it	Not at All Intense				Very Intense
0	**1**	**2**	**3**	**4**	**5**

c. At the end of the day, within two hours of going to bed?

Never have it	Not at All Intense				Very Intense
0	**1**	**2**	**3**	**4**	**5**

d. When your eyes felt burning and stinging, **how much did the burning and stinging bother you**?

Never have it	Not at All bothered				Extremely bothered
0	**1**	**2**	**3**	**4**	**5**

10. Questions about **TIRED EYES:**

a. During a typical day in the past week, **how often** did your eyes feel tired?

0 Never
1 Rarely
2 Sometimes
3 Frequently
4 Constantly

When your eyes felt tired, **how intense was this feeling of tired eyes** …

b. Within the first two hours of getting up in the morning?

Never have it	Not at All Intense				Very Intense
0	**1**	**2**	**3**	**4**	**5**

c. At the end of the day, within two hours of going to bed?

Never have it	Not at All Intense				Very Intense
0	**1**	**2**	**3**	**4**	**5**

d. When your eyes felt tired, **how much did the feeling of tired eyes bother you**?

Never have it	Not at All bothered				Extremely bothered
0	**1**	**2**	**3**	**4**	**5**

11. Questions about **CHANGEABLE, BLURRY VISION:**

a. During a typical day in the past week, **how often** did your vision change between clear and blurry or foggy?

0 Never
1 Rarely
2 Sometimes
3 Frequently
4 Constantly

When your vision was blurry, **how noticeable was the changeable, blurry, or foggy vision** …

b. Within the first two hours of getting up in the morning?

Never have it	Not at All Noticeable				Very Noticeable
0	**1**	**2**	**3**	**4**	**5**

c. At the end of the day, within two hours of going to bed?

Never have it	Not at All Intense				Very Intense
0	**1**	**2**	**3**	**4**	**5**

d. When your vision was blurry, **how much did the changeable, blurry or foggy vision bother you**?

Never have it	Not at All bothered				Extremely bothered
0	**1**	**2**	**3**	**4**	**5**

12. Question about **EYELID REDNESS:**

During a typical day in the past week, **how often** did your eyelid margins look red?

0 Never
1 Rarely
2 Sometimes
3 Frequently
4 Constantly

13. Question about **WATERY EYES:**

During a typical day in the past week, **how often** did your eyes look or feel excessively watery?

0 Never
1 Rarely
2 Sometimes
3 Frequently
4 Constantly

14. Question about **EYE MUCUS AND CRUSTING:**

During a typical day in the past week, **how often** was mucus or crusty material in or around your eyes?

0 Never
1 Rarely
2 Sometimes
3 Frequently
4 Constantly

15. Question about **CLOSING YOUR EYES:**

During a typical day in the past week, **how often** did your eyes bother you so much that you wanted to close them?

0 Never
1 Rarely
2 Sometimes
3 Frequently
4 Constantly

16. Questions about how much different **TYPES OF AIR QUALITY BOTHER YOUR EYES:**

a. a room with **cigarette smoke or smog**?

Never have it	Not at all				Very much
0	**1**	**2**	**3**	**4**	**5**

b. a building with the **central air conditioning or heating** turned on?

Never have it	Not at all				Very much
0	**1**	**2**	**3**	**4**	**5**

c. **shopping at the mall** or **shopping in retail or fabric stores**?

Never have it	Not at all				Very much
0	**1**	**2**	**3**	**4**	**5**

17. Question about **ARTIFICIAL TEAR USE:**

During a typical day in the past week, **how often** did you use artificial tears?

0 Never
1 Rarely
2 Sometimes
3 Frequently
4 Constantly

18. Question about **DRYNESS OF THE NOSE, MOUTH, OR VAGINA:**

During a typical day in the past week, **how often** did you experience dryness of the nose, mouth, or vagina?

0 Never
1 Rarely
2 Sometimes
3 Frequently
4 Constantly

19. During a typical day in the past week, **how often did you use a computer**?

0 Never
1 1 to 2 hours
2 3 to 6 hours
3 More than 6 hours

20. Are you currently taking any of the following medications?

		Yes	No
a.	Thyroid medications	**1**	**2**
b.	Blood pressure medications	**1**	**2**
c.	Diabetes medications	**1**	**2**
d.	Diuretics	**1**	**2**
e.	Arthritis medications	**1**	**2**
f.	Heart condition medications	**1**	**2**
g.	Depression medications	**1**	**2**
h.	Ulcer medications	**1**	**2**
i.	Oral contraceptives	**1**	**2**
j.	Antibiotics for acne or other skin conditions	**1**	**2**
k.	Hormone replacement therapy	**1**	**2**
l.	Allergy medications	**1**	**2**

21. Have you been told you have dry eye(s)?

1 Yes **2** No

22. If you use any of the following treatments for dry eye, how much help do they provide?

		No help At all				Complete Relief	Do Not Use
a.	Artificial tears	**1**	**2**	**3**	**4**	**5**	**0**
b.	Lubricating ointments or gels	**1**	**2**	**3**	**4**	**5**	**0**
c.	Warm compresses or eyelid scrubs	**1**	**2**	**3**	**4**	**5**	**0**
d.	Punctal plugs or cauterization	**1**	**2**	**3**	**4**	**5**	**0**
e.	Room humidifier	**1**	**2**	**3**	**4**	**5**	**0**
f.	Other (please specify below)	**1**	**2**	**3**	**4**	**5**	**0**

_____

23. Do you think you have dry eye(s)?

1 Yes **2** No

THANK YOU VERY MUCH!

APPENDIX E DRY EYE QUESTIONNAIRE 5 (DEQ 5)

DEQ 5

1. Questions about **EYE DISCOMFORT**:
   a. During a typical day in the past month, **how often** did your eyes feel discomfort?

      0 Never
      1 Rarely
      2 Sometimes
      3 Frequently
      4 Constantly

   b. When your eyes felt discomfort, **how intense was this feeling of discomfort** at the end of the day, within two hours of going to bed?

Never have it	Not at All Intense				Very Intense
0	**1**	**2**	**3**	**4**	**5**

2. Questions about **EYE DRYNESS**:
   a. During a typical day in the past month, **how often** did your eyes feel dry?

      0 Never
      1 Rarely
      2 Sometimes
      3 Frequently
      4 Constantly

   b. When your eyes felt dry, **how intense was this feeling of dryness** at the end of the day, within two hours of going to bed?

Never have it	Not at All Intense				Very Intense
0	**1**	**2**	**3**	**4**	**5**

3. Question about **WATERY EYES:**
   During a typical day in the past month, **how often** did your eyes look or feel excessively watery?

      0 Never
      1 Rarely
      2 Sometimes
      3 Frequently
      4 Constantly

Score: 1a + 1b + 2a + 2b + 3 = Total
___+___+___+___+___=___

APPENDIX F NUMERICAL RATING SCORE QUESTIONNAIRE (NRS)

PLEASE RATE THE FOLLOWING SENSATION DURING A TYPICAL DAY IN THE LAST WEEK ON A SCALE FROM 1-100, WHERE:

1 = SEVERE SENSATION

100 = "PERFECT" OR NO SENSATION AT ALL.

SYMPTOM	SCORE
COMFORT	
DRYNESS	
FOREIGN BODY SENSATION	
BURNING	
WATERING	

APPENDIX GSUBJECTIVE EVALUATION OF SYMPTOMS OF DRYNESS(SESoD)

PLEASE EVALUATE YOUR OCULAR DISCOMFORT DUE TO THE SYMPTOM OF "DRYNESS" ON A SCALE OF 0 (NONE) TO 4 (SEVERE)	

YOU MAY USE THE FOLLOWING DESCRIPTIONS TO ASSIST IN YOUR SCORE:

NONE (0) I DO NOT HAVE THIS SYMPTOM

TRACE (1) I SELDOM NOTICE THIS SYMPTOM, AND IT DOES NOT MAKE ME UNCOMFORTABLE

MILD (2)I SOMETIMES NOTICE THIS SYMPTOM, IT DOES MAKE ME UNCOMFORTABLE, BUT IT DOES NOT INTERFERE WITH MY ACTIVITIES

MODERATE (3) I FREQUENTLY NOTICE THIS SYMPTOM, IT DOES MAKE ME UNCOMFORTABLE, AND IT SOMETIMES INTERFERES WITH MY ACTIVITIES

APPENDIX H MENOPAUSE-SPECIFIC QUALITY OF LIFE QUESTIONNAIRE (MENQOL)

INSTRUCTION: For each of the following item indicate whether you have experienced theproblem in the PAST WEEK.

- If NO, tick NO then move onto the next question
- If YES, tick YES **and** circle a number to show how bothered you were by the problem

This questionnaire is completely confidential and your name will not be associated with your responses. If, however for any reason you do not wish to complete an item, please leave it and go on to the next one

	Not bothered → extremely bothered	
Hot flushes	NO □	YES □ 0 1 2 3 4 5 6
2. Night sweats	NO □	YES □ 0 1 2 3 4 5 6
3. Sweating	NO □	YES □ 0 1 2 3 4 5 6
4. Being dissatisfied with my personal life	NO □	YES □ 0 1 2 3 4 5 6
5. Feeling anxious or nervous	NO □	YES □ 0 1 2 3 4 5 6
6. Experiencing poor memory	NO □	YES □ 0 1 2 3 4 5 6
7. Accomplishing less than I used to	NO □	YES □ 0 1 2 3 4 5 6
8. Feeling depressed, down or blue	NO □	YES □ 0 1 2 3 4 5 6
9. Being impatient with other people	NO □	YES □ 0 1 2 3 4 5 6
10. Feelings of wanting to be alone	NO □	YES □ 0 1 2 3 4 5 6
11. Flatulence or gas pains	NO □	YES □ 0 1 2 3 4 5 6
12. Aching in muscles and joints	NO □	YES □ 0 1 2 3 4 5 6
13. Feeling tired or worn out	NO □	YES □ 0 1 2 3 4 5 6
14. Difficulty sleeping	NO □	YES □ 0 1 2 3 4 5 6
15. Aches in back of head or neck	NO □	YES □ 0 1 2 3 4 5 6

Not bothered → extremely bothered

16. Decrease in physical strength	NO □	YES □	0	1	2	3	4	5	6
17. Decrease in stamina	NO □	YES □	0	1	2	3	4	5	6
18. Feeling a lack of energy	NO □	YES □	0	1	2	3	4	5	6
19. Drying skin	NO □	YES □	0	1	2	3	4	5	6
20. Weight gain	NO □	YES □	0	1	2	3	4	5	6
21. Increased facial hair	NO □	YES □	0	1	2	3	4	5	6
22. Changes in appearance in texture or tone of your skin	NO □	YES □	0	1	2	3	4	5	6
23. Feeling bloated	NO □	YES □	0	1	2	3	4	5	6
24. Low backache	NO □	YES □	0	1	2	3	4	5	6
25. Frequent urination	NO □	YES □	0	1	2	3	4	5	6
26. Involuntary urination when laughing or coughing	NO □	YES □	0	1	2	3	4	5	6
27. Change in your sexual desire	NO □	YES □	0	1	2	3	4	5	6
28.Involuntary urination when laughing or coughing	NO □	YES □	0	1	2	3	4	5	6
29. Avoiding intimacy	NO □	YES □	0	1	2	3	4	5	6

Domains:

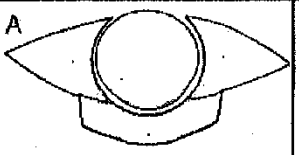
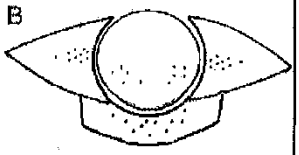
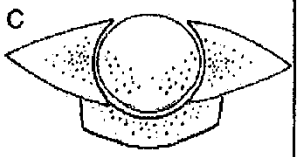
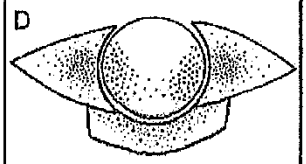
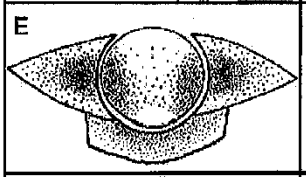
Vasomotor: sum items 1,2 and 3
Psychosocial: sum items 4-10
Physical: sum items 11-26
Sexual: sum items 27-29

University of Toronto

APPENDIX I MODIFIED OXFORD SCALE

Corneal and conjunctival staining

GRADING OF CORNEAL AND CONJUNCTIVAL STAINING
MODIFIED OXFORD SCHEME

PANEL	GRADE	VERBAL DESCRIPTOR
A 	0	Absent Dot Count: 1 (per sector)
B 	I	Minimal Dot Count: 10 (per sector)
C 	II	Mild Dot Count: 32 (per sector)
D 	III	Moderate Dot Count: 100 (per sector)
E 	IV	Marked Dot Count: 316 (per sector)
>E	V	Severe (Dot Count >316)

Appendix J SOP for Meibomian Gland and Eyelid Grading

(1) Take a high magnification photo (40X) of central lid margin. The glands should be in sharp focus on the lower lid (This photo will be used for counting the gland)

(2) Calculate Marx's line score

(3) Perform lid grading at 16x magnification

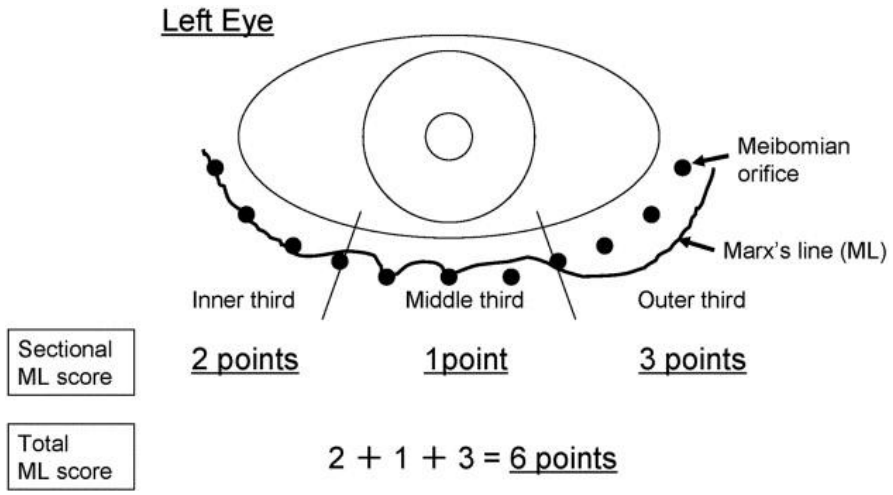
(4) Check expressibility and secretion quality last

Acini (assess full length of the tarsal conjunctiva)

Concretions	Absent 0	Deep 1	Subepithelial 2	Extruding 3
Chalazia	Absent 0	Deep 1	Subepithelial 2	Extruding 3

Marx's line (assess after instilling lissamine green using the Yamaguchi grading scale below- instil lissamine green on the upper temporal conjunctiva if using strips). Divide the lid margin into 3 equal sections (inner, middle and outer) as shown in the figure below and grade each section according to the table below. The final score is the sum of the 3 scores. The highest possible score is 9. The lower lid is scored separately to the upper lid

0	ML runs entirely along the conjunctival side of the Meibomian orifices
1	Parts of ML touch the Meibomian orifices
2	ML pass through the orifices
3	ML runs on the eyelid margin side of the meibomian orifices



Yamaguchi et al (2006) American Journal of Ophthalmology 141: 669-69.e8

Retroplaced Orifices (The orifices which have moved to the conjunctival side of the lid margin **-** assess the entire length of lid with lissamine green**)**

Absent 0	<1mm behind Marx's line 1	1-2mm behind Marx's line 2	>2mm behind Marx's line 3

Lid Margin (assess full length of lid)

Notching	Absent 0		Present 1	
Rounding	Absent 0		Present 1	
Hyperkeritanization	Absent 0		Present 1	
Vascularity	None 0	Mild 1	Moderate 2	Severe 3
Telangiectasia (count number)	None 0	Single 1	Greater than 2 2	Greater than 5 3

Orifices (assess full length of lid)

Capping- count how many	Number	
Pouting	Absent 0	Present 1
Narrowing- invisible punctum	Absent 0	Present 1
Scarring	Absent 0	Present 1

Meibomian gland Expressibility: Character of secretion expressed (assess central 10 glands) of lower lid

Secretion expression	Minimal pressure 0	Light pressure 1	Moderate pressure 2	Heavy pressure 3	Not expressible 4
Secretion quality	Clear 0	Cloudy 1	Granular 2	Solid 3	

Number of glands patent (assess central 10 glands) of lower lid

Count how many express oil	Count number

Orifices (assess central part of the lid)of lower lid

Number –count how many appear in photo 40x	number

Acronyms written in the tables K TO T

E2: oestradiol
Diol G: 3 α-diol G
DHEA-S: DHEA-S: Dehydroepiandrosterone Sulfate
E2:Dg: Ratio between oestradiol and 3 α-diol G
OSDI: Ocular Surface Index
OCI: Ocular Comfort Index
Oci_Fre: Ocular Comfort Index frequency
Oci_ Int: Ocular Comfort Index intensity
Vasomotor domain
Psychosocial domain
Physical domain
Sexual domain
Tear Osmolarity
PRT: Phenol red thread
Sch: Schimer
CS: Corneal sensitivity
ICJS: Inferior conjunctival sensitivity
CSt: Corneal staining
ICJSt: Inferior Conjunctival Staining
Nogla: Number of glands
Nocap: Number of capped glands
Expressibility: Meibomian gland expressibility
Marx: Marx Line score

Appendix K: The Correlation coefficient values (r) and p values of the associations between sex hormone concentrations, ratio of oestradiol to androgens and ocular surface sensitivity and dry eye clinical signs in all subjects Red figures indicate significance level at $p < 0.05$ while blue figures indicate $0.05 < p < 0.25$

Variables		Oestradiol	Progesterone	Total Testosterone	Free Testosterone	DHEAS	3α-diol G	SHBG	Oestradiol: Total T	Oestradiol: 3α-diol G
Central corneal sensitivity	Correlation Coefficient	.075	-.041	.210	.187	.223	.276*	-.107	-0.130	-0.170
	Sig. (2tailed)	.524	.729	.072	.110	.057	.017	.364	0.262	0.142
Inferior conjunctival Sensitivity	Correlation Coefficient	-.012	.056	.046	.043	.046	.177	.034	-0.034	-0.144
	Sig. (2-tailed)	.919	.638	.699	.714	.697	.131	.777	0.769	0.214
Tear volume	Correlation Coefficient	-.041	.017	.072	.193	.280	.350	-.170	-0.105	-0.307
	Sig. (2-tailed)	.731	.888	.547	.102	.002	.003	.150	0.366	0.007
Tear osmolarity	Correlation Coefficient	.069	-.193	-.227	-.152	-.312**	-.147	.039	0.275	0.199
	Sig. (2-tailed)	.570	.106	.057	.207	.002	.222	.744	0.016	0.085
NIBUT	Correlation Coefficient	-.189	-.064	.101	.091	.260	.133	-.038	-0.032	-0.132
	Sig. (2-tailed)	.107	.588	.390	.442	.00 3	.258	.746	0.784	0.255
Corneal Staining	Correlation Coefficient	.162	-.025	.002	-.049	-.074	-.063	.026	0.152	-0.006
	Sig. (2-tailed)	.173	.835	.987	.686	.535	.599	.831	0.189	0.959
Conjunctival Staining	Correlation Coefficient	.041	-.012	.060	.002	.222	-.100	.012	-0.006	0.121
	Sig. (2-tailed)	.727	.921	.612	.984	.058	.395	.920	0.959	0.297
Marx's Line	Correlation Coefficient	-.079	-.021	-.059	.076	-.079	-.005	-.030	0.014	-0.019
	Sig. (2-tailed)	.502	.861	.617	.518	.503	.965	.802	0.902	0.869

Appendix K The Correlation coefficient values r) and p values of the associations between sex hormone concentrations, ratio of oestradiol to androgens and ocular surface sensitivity and dry eye clinical signs in all subjects (continuation). Red figures indicate significance level at $p < 0.05$ while blue figures indicate $0.05 < p < 0.25$

Variables		Oestradiol	Progesterone	Total Testosterone	Free Testosterone	DHEAS	3α-diol G	SHBG	Oestradiol: Total T	Oestradiol: 3α-diol G
Meibomian gland expressibility	Correlation Coefficient	.054	.011	-.091	-.090	.034	-.050	.037	0.126	0.086
	Sig. (2-tailed)	.648	.924	.442	.449	.773	.674	.755	0.277	0.460
Number of glands	Correlation Coefficient	.245	-.008	-.030	-.050	.008	-.016	.046	0.187	0.127
	Sig. (2-tailed)	.051	.952	.814	.694	.052	.901	.721	0.106	0.275
Number of patent glands	Correlation Coefficient	.162	.147	-.093	-.107	.006	.026	-.010	0.169	0.074
	Sig. (2-tailed)	.169	.212	.429	.364	.610	.829	.930	0.144	0.526
Number of capped glands	Correlation Coefficient	-.072	-.031	-.090	-.108	.015	-.116	.150	0.014	0.065
	Sig. (2-tailed)	.543	.794	.444	.360	.898	.323	.201	0.903	0.579

Appendix L The Correlation coefficient values (r) and p values of the associations between sex hormone concentrations, ratio of oestradiol to androgens and ocular surface sensitivity and dry eye clinical indices in females. Red figures indicate significance level at p< 0.05 while blue figures indicate 0.05 <p < 0.25

Variables		Oestradiol	Progesterone	Total Testosterone	Free Testosterone	DHEAS	3α-diol G	SHBG	Oestradiol: Total Testosterone	Oestradiol: 3α-diol G
Central corneal sensitivity	Correlation Coefficient	.138	.002	-.005	-.082	.175	.140	.167	.903	-.013
	Sig. (2-tailed)	.326	.991	.973	.561	.210	.317	.233	.502	.926
Inferior conjunctival Sensitivity	Correlation Coefficient	.006	.083	-.016	-.014	-.019	.182	.154	.012	-.159
	Sig. (2-tailed)	.964	.554	.910	.920	.893	.191	.272	.930	.251
Tear volume	Correlation Coefficient	-.068	.020	-.246	-.033	-.195	.308*	-.001	.082	.076
	Sig. (2-tailed)	.632	.887	.079	.816	.166	.026	.997	.556	.586
Tear osmolarity	Correlation Coefficient	-.010	-.272	-.129	-.014	-.334	-.147	.039	.166	-.198
	Sig. (2-tailed)	.942	.056	.371	.926	.021	.222	.744	.229	.152
NBUT	Correlation Coefficient	-.255	-.029	-.098	-.050	-.150	.133	-.038	.047	.280
	Sig. (2-tailed)	.065	.837	.483	.724	.226	.258	.746	.738	.040
Corneal Staining	Correlation Coefficient	.152	-.122	.094	-.032	.035	.029	-.037	.113	.078
	Sig. (2-tailed)	.287	.394	.510	.821	.806	.839	.795	.415	.574
Conjunctival Staining	Correlation Coefficient	.106	.005	.169	.096	.349*	-.141	-.141	.017	.186
	Sig. (2-tailed)	.449	.969	.227	.496	.010	.312	.314	.902	.177
Marx's Line	Correlation Coefficient	-.204	-.062	.175	.400**	.101	.138	-.248	.286	.241
	Sig. (2-tailed)	.144	.659	.211	.011	.470	.323	.073	.036	.079

Appendix L The Correlation coefficient values (r) and p values of the associations between sex hormone concentrations and ocular surface sensitivity and dry eye clinical indices in females (Continuation). Red figures indicate significance level at p< 0.05 while blue figures indicate 0.05 <p < 0.25

Variables		Oestradiol	Progesterone	Total Testosterone	Free Testosterone	DHEAS	3α-diol G	SHBG	Oestradiol: Total Testosterone	Oestradiol: 3α-diol G
Meibomian gland expressibility	Correlation Coefficient	.135	.080	-.091	-.121	-.036	.000	.096	.157	.127
	Sig. (2-tailed)	.341	.574	.522	.393	.802	1.000	.498	.258	.360
Number of glands	Correlation Coefficient	.352*	-.028	.031	-.083	.125	.007	.135	.032	.072
	Sig. (2-tailed)	.015	.850	.835	.577	.402	.960	.364	.888	.749
Number of patent glands	Correlation Coefficient	.106	.030	-.034	-.108	.004	.051	-.018	.157	.090
	Sig. (2-tailed)	.450	.830	.809	.440	.978	.715	.898	.258	.518
Number of capped glands	Correlation Coefficient	-.051	.012	-.005	-.058	.061	-.033	.173	.109	.002
	Sig. (2-tailed)	.717	.932	.974	.680	.664	.812	.215	.434	.989

Appendix M The Correlation coefficient values (r) and p values of the associations between sex hormone concentrations and ocular surface sensitivity and dry eye clinical indices in males. Red figures indicate significance level at p< 0.05 while blue figures indicate 0.05 <p< 0.25

Variables		Oestradiol	Progesterone	Total Testosterone	Free Testosterone	DHEAS	3α-diol G	SHBG	Oestradiol: Total Testosterone	Oestradiol: 3α-diol G
Central corneal sensitivity	Correlation Coefficient	.150	.169	.085	.518*	.190	.019	-.549*	-.150	-.083
	Sig. (2-tailed)	.515	.465	.713	.017	.409	.934	.010	.506	.715
Inferior conjunctival Sensitivity	Correlation Coefficient	-.152	-.118	.243	.320	.095	-.079	-.233	.366	-.223
	Sig. (2-tailed)	.511	.611	.289	.158	.681	.732	.309	.094	.318
Tear volume	Correlation Coefficient	.291	.515*	.000	.218	.361	.412	-.198	.236	-.034
	Sig. (2-tailed)	.201	.017	1.000	.343	.107	.063	.390	.291	.881
Tear osmolarity	Correlation Coefficient	.107	-.292	.071	-.027	-.227	-.020	.205	.229	.290
	Sig. (2-tailed)	.643	.199	.759	.908	.322	.931	.373	.305	.190
NBUT	Correlation Coefficient	.357	.109	.190	.232	-.078	-.107	.147	.285	.352
	Sig. (2-tailed)	.112	.638	.410	.312	.736	.645	.524	.199	.109
Corneal Staining	Correlation Coefficient	.093	.226	.327	.137	-.275	-.141	-.004	.009	.180
	Sig. (2-tailed)	.688	.325	.148	.554	.227	.541	.985	.968	.422
Conjunctival Staining	Correlation Coefficient	-.202	-.170	-.055	-.279	-.035	-.056	.312	.098	.092
	Sig. (2-tailed)	.381	.460	.812	.221	.881	.808	.168	.665	.682
Marx's Line	Correlation Coefficient	.195	-.296	-.147	-.160	-.430	-.041	.302	.150	.197
	Sig. (2-tailed)	.397	.193	.526	.489	.0047	.860	.183	.504	.381

Appendix M The Correlation coefficient values (r) and p values of the associations between sex hormone concentrations and ocular surface sensitivity and dry eye clinical indices in males (continuation). Red figures indicate significance level at $p < 0.05$ while blue figures indicate $0.05 < p < 0.25$

Variables		Oestradiol	Progesterone	Total Testosterone	Free Testosterone	DHEAS	3α-diol G	SHBG	Oestradiol: Total Testosterone	Oestradiol: 3α-diol G
Meibomian gland expressibility	Correlation Coefficient	-.136	-.214	-.353	-.152	.193	-.110	-.225	.224	-0.170
	Sig. (2-tailed)	.556	.351	.116	.512	.401	.635	.327	.317	.939
Number of glands	Correlation Coefficient	-.092	.056	-.111	.107	.048	-.329	-.273	.032	.072
	Sig. (2-tailed)	.727	.830	.671	.684	.854	.197	.289	..888	.749
Number of patent glands	Correlation Coefficient	.194	.489*	-.022	.095	.242	.621*	-.239	.339	.138
	Sig. (2-tailed)	.399	.024	.925	.682	.291	.0004	.296	.123	.541
Number of capped glands	Correlation Coefficient	-.200	-.273	-.285	-.261	-.161	-.216	.120	..089	.131
	Sig. (2-tailed)	.385	.231	.210	.253	.486	.346	.604	.692	.560

Appendix N The Correlation coefficient values (r) and p values of the associations between sex hormone concentrations, ratio of oestradiol to androgens and ocular symptoms, age and contact lens wear and in all subjects. Red figures indicate significance level at p< 0.05 while blue figures indicate 0.05 <p < 0.25

Ocular Symptoms		CL Wear	Age	Oestradiol	Progesterone	Total Testosterone	Free Testosterone	DHEAS	3α-diol G	SHBG	Oestradiol: Total T	Oestradiol: 3α-diol G
OSDI	Correlation	-.037	.053	.130	-.106	-.261	-.342	-.277*	.005	-.044	0.319	0.228
	Sig.	.791	.708	.359	.456	.003	.003	.047	.970	.755	0.007	0.047
OCI	Correlation	.128	-.103	.136	-.040	-.009	-.262	-.092	-.046	.092	0.193	0.187
	Sig.	.356	.460	.331	.774	.950	.002	.514	.742	.513	0.094	0.136
SESOD	Correlation	.283*	-.109	.215	-.004	.079	-.040	.199	.069	-.073	0.110	0.116
	Sig.	.038	.432	.122	.978	.573	.777	.153	.624	.603	0.346	0.318
DEQ5	Correlation	.314*	-.203	.243	.039	.114	-.101	.015	-.032	.046	0.188	0.191
	Sig.	.021	.141	.004	.779	.417	.471	.913	.820	.745	0.105	0.098
DEQ Frequency	Correlation	.089	-.024	.203	.017	.005	-.0.261	-.101	.042	.080	0.241	0.138
	Sig.	.523	.866	.145	.901	.971	.001	.473	.765	.568	0.036	0.235
DEQ Intensity	Correlation	.213	-.104	.196	.003	.106	-.070	.032	-.023	-.012	0.028	0.018
	Sig.	.122	.453	.160	.984	.449	.618	.819	.871	.934	0.810	0.878
DEQ Intensity A.M	Correlation	.104	-.003	.058	.004	.017	-.019	.005	.039	-.041	0.196	0.146
	Sig.	.455	.982	.680	.977	.903	.895	.972	.783	.772	0.089	0.210
DEQ Intensity P.M	Correlation	.229	-.110	.226	.004	.094	-.088	-.001	-.019	-.012	0.201	0.185
	Sig.	.096	.426	.104	.975	.502	.529	.996	.890	.929	0.081	0.109

Appendix N The Correlation coefficient values (r) and p values of the associations between sex hormone concentrations, ratio of oestradiol to androgens and ocular symptoms, age and contact lens wear and in all subjects (continuation). Red figures indicate significance level at $p < 0.05$ while blue figures indicate $0.05 < p < 0.25$

Ocular Symptoms		CL Wear	Age	Oestradiol	Progesterone	Total Testosterone	Free Testosterone	DHEAS	3α-diol G	SHBG	Oestradiol: Total Testosterone	Oestradiol: 3α-diol G
DEQ Bother	Correlation	.207	-.033	.164	.011	.006	-.262	-.042	-.027	-.060	0.196	0.146
	Sig.	.133	.813	.241	.937	.967	.002	.765	.848	.667	0.089	0.210
DEQ Total	Correlation	.173	-.055	.175	.008	.039	-.127	-.052	-.003	-.006	0.220	0.165
	Sig.	.210	.694	.209	.953	.780	.365	.713	.981	.966	0.057	0.181
NRS Comfort	Correlation	-.257	.131	-.109	-.059	.094	.196	-.030	.146	-.014	-0.039	-0.038
	Sig.	.063	.351	.441	.678	.507	.165	.835	.301	.919	0.741	0.746
NRS Dryness	Correlation	-.321*	.142	-.310*	-.052	-.115	.118	-.181	.017	-.050	-0.148	-0.126
	Sig.	.018	.307	.024	.713	.412	.400	.194	.906	.725	0.201	0.276
NRS Foreign Body	Correlation	-.225	.131	-.328*	-.054	-.276*	-.046	-.184	-.058	-.070	-0.131	-0.147
	Sig.	.106	.350	.018	.704	.047	.745	.191	.685	.620	0.259	0.206
NRS Burning	Correlation	-.110	-.026	-.257	-.072	-.135	.079	-.127	.055	.001	-0.191	-0.177
	Sig.	.434	.856	.066	.614	.340	.576	.369	.699	.996	0.099	0.136
NRS Watery	Correlation	-.054	.055	-.045	.232	.023	.043	.039	.019	-.039	-0.053	0.001
	Sig.	.703	.697	.753	.098	.874	.763	.785	.895	.783	0.649	0.995

Appendix O The Correlation coefficient values (r) and p values of the associations between sex hormone concentrations and ocular symptoms, age and contact lens wear in females. Red figures indicate significance level at p< 0.05 while blue figures indicate 0.05 <p < 0.25

Ocular Symptoms		CL Wear	Age	Oestradiol	Progesterone	Total Testosterone	Free Testosterone	DHEAS	3α-diol G	SHBG	Oestradiol: Total Testosterone	Oestradiol: 3α-diol G
OSDI	Correlation	-.316	-.161	-.121	.085	.139	-.074	.044	.293	.163	.301	.165
	Sig.	.152	.475	.601	.714	.549	.751	.851	.198	.479	.027	.232
OCI	Correlation	-.162	-.177	-.045	.057	.273	.035	.124	.248	.195	.163	.152
	Sig.	.471	.430	.845	.807	.232	.881	.591	.277	.398	.239	.272
SESOD	Correlation	.429*	-.178	-.163	-.077	.193	.064	.086	.061	.191	.104	.907
	Sig.	.046	.428	.481	.739	.401	.784	.710	.794	.406	.455	.487
DEQ5	Correlation	.279	-.116	-.159	-.041	-.054	-.341	.065	.228	.096	.206	.249
	Sig.	.209	.606	.491	.861	.815	.131	.780	.320	.679	.135	.070
DEQ Frequency	Correlation	-.247	-.065	-.239	-.136	-.142	-.189	-.050	.258	.100	.236	.123
	Sig.	.268	.774	.296	.555	.539	.412	.829	.258	.665	.085	0.375
DEQ Intensity	Correlation	-.146	.048	-.123	-.100	-.112	-.373	.052	.310	.178	.164	.167
	Sig.	.515	.834	.594	.667	.627	.096	.824	.172	.441	.236	.175
DEQ Intensity A.M	Correlation	-.260	.161	-.065	-.010	-.022	-.321	-.072	.392	.356	.080	.073
	Sig.	.243	.475	.781	.967	.925	.156	.755	.078	.113	.566	.602
DEQ Intensity P.M	Correlation	-.085	-.036	-.134	-.129	-.141	-.342	.104	.224	.064	.179	.182
	Sig.	.706	.872	.562	.577	.543	.129	.653	.329	.781	.195	.187
DEQ Bother	Correlation	-.101	.188	-.212	-.295	-.243	-.465*	-.002	.218	.207	.161	.110
	Sig.	.655	.401	.356	.195	.288	.034	.994	.342	.368	.245	.430

Appendix OThe Correlation coefficient values (r) and p values of theassociations between sex hormone levels and ocular symptoms, age and contact lens wear in females (continuation). Red figures indicate significance level at p< 0.05 while blue figures indicate 0.05 <p < 0.25

Ocular Symptoms		CL Wear	Age	Oestradiol	Progesterone	Total Testosterone	Free Testosterone	DHEAS	3α-diol G	SHBG	Oestradiol: Total Testosterone	Oestradiol: 3α-diol G
DEQ Total	Correlation	-.200	.066	-.235	-.212	-.230	-.440*	.039	.266	.172	.183	.125
	Sig.	.372	.770	.304	.357	.315	.046	.867	.245	.457	.186	.368
NRS Comfort	Correlation	-.094	.209	.148	.125	-.039	.208	-.103	-.255	-.267	-.196	-.165
	Sig.	.678	.351	.523	.588	.866	.365	.658	.265	.241	155	232
NRS Dryness	Correlation	-.336	.105	.328	.217	.223	.129	-.009	-.330	.025	-.210	-.203
	Sig.	.127	.641	.002	.344	.331	.578	.968	.144	.914	127	.141
NRS Foreign Body	Correlation	-.599**	.351	.331	-.210	.152	.166	-.335	-.269	-.051	-.064	-.139
	Sig.	.003	.109	.002	.362	.511	.472	.138	.239	.827	.646	.317
NRS Burning	Correlation	0.000	.108	.111	-.020	.310	.423	-.202	-.177	-.101	-.160	-.187
	Sig.	1.000	.632	.631	.931	.171	.056	.380	.442	.662	.249	.175
NRS Watery	Correlation	-.219	.364	.061	-.097	.028	-.050	-.108	-.450*	.008	-.089	-.012
	Sig.	.328	.096	.791	.677	.903	.829	.640	.041	.973	.523	.930

Appendix P The Correlation coefficient values (r) and p values of the associations between ocular symptoms and ocular surface sensitivity, integrity and tear function in all subjects. Red figures indicate significance level at $p < 0.05$ while blue figures indicate $0.05 < p < 0.25$

Ocular Symptoms		Corneal Sensitivity	Inferior Conjunctival Sensitivity	Tear Osmolarity	Tear Volume	NBUT	Corneal Staining	Conjunctival Staining
OSDI	Correlation	-.043	-.065	.187	-.080	.054	.031	-.100
	Sig.	.717	.581	.115	.496	.647	.796	.395
OCI	Correlation	.110	-.018	.140	-.131	-.114	.061	.079
	Sig.	.343	.879	.238	.263	.327	.603	.496
SESOD	Correlation	.197	.052	-.115	-.200	-.117	.110	.395**
	Sig.	.087	.654	.331	.085	.314	.350	.000
DEQ5	Correlation	.109	-.141	.082	-.295*	-.188	.220	.314**
	Sig.	.347	.225	.489	.010	.104	.060	.006
DEQ Frequency	Correlation	.055	-.114	.171	-.189	-.106	.097	.128
	Sig.	.638	.329	.148	.105	.364	.411	.270
DEQ Intensity	Correlation	.081	-.071	.173	-.132	-.069	.055	.040
	Sig.	.484	.545	.142	.258	.556	.639	.733
DEQ Intensity A.M	Correlation	-.065	-.071	.172	-.043	.009	.118	.133
	Sig.	.575	.544	.145	.713	.940	.317	.252
DEQ Intensity P.M	Correlation	.101	-.115	.130	-.327**	-.114	.182	.202
	Sig.	.385	.321	.272	.004	.328	.121	.080
DEQ Bother	Correlation	.048	-.128	.168	-.259*	-.073	.182	.177
	Sig.	.682	.270	.155	.025	.529	.120	.127

Appendix P The Correlation coefficient values (r) and p values of the associations between ocular symptoms and ocular surface sensitivity, integrity and tear function in all subjects (continuation). Red figures indicate significance level at p< 0.05 while blue figures indicate0.05 <p < 0.25

Ocular Symptoms		Corneal Sensitivity	Inferior Conjunctival Sensitivity	Tear Osmolarity	Tear Volume	NBUT	Corneal Staining	Conjunctival Staining
DEQ Total	Correlation	.036	-.124	.168	-.277*	-.167	.103	.162
	Sig.	.757	.285	.155	.016	.148	.383	.163
NRS Comfort	Correlation	-.106	.093	-.031	.148	.063	-.197	-.354**
	Sig.	.367	.430	.795	.209	.592	.095	.002
NRS Dryness	Correlation	-.157	-.091	.041	.133	.224	-.187	-.354**
	Sig.	.177	.436	.728	.256	.052	.110	.002
NRS Foreign Body	Correlation	-.144	-.043	.142	.149	.110	-.130	-.234*
	Sig.	.218	.712	.235	.205	.346	.274	.043
NRS Burning	Correlation	-.062	-.020	.034	.149	.079	.000	-.201
	Sig.	.597	.863	.778	.206	.500	.998	.083
NRS Watery	Correlation	-.065	-.002	-.073	-.129	.031	-.157	-.013
	Sig.	.582	.990	.544	.273	.793	.184	.915

Appendix Q The Correlation coefficient values (r) and p values of the associations between ocular symptoms and ocular surface sensitivity, integrity and tear function in females. Red figures indicate significance level at p< 0.05 while blue figures indicate0.05 <p < 0.25

Ocular Symptoms		Corneal Sensitivity	Inferior Conjunctival Sensitivity	Tear Osmolarity	Tear Volume	NBUT	Corneal Staining	Conjunctival Staining
OSDI	Correlation	-.030	-.227	.161	.082	.035	.020	-.161
	Sig.	.834	.102	.264	.565	.803	.891	.251
OCI	Correlation	.143	-.178	-.041	-.072	-.280	.099	.055
	Sig.	.301	.198	.774	.608	.040	.484	.690
SESOD	Correlation	.239	-.061	-.150	-.103	-.153	.078	.364**
	Sig.	.082	.663	.292	.462	.268	.581	.007
DEQ5	Correlation	.183	-.292*	.058	.232	-.272*	.263	.245
	Sig.	.186	.032	.686	.094	.047	.059	.074
DEQ Frequency	Correlation	.139	-.235	.149	-.118	-.247	.094	.098
	Sig.	.315	.088	.298	.401	.072	.509	.479
DEQ Intensity	Correlation	.151	-.205	.155	-.062	-.225	.046	.020
	Sig.	.276	.138	.277	.657	.102	.743	.888
DEQ Intensity A.M	Correlation	.008	-.085	.104	-.045	-.025	.119	.080
	Sig.	.953	.540	.469	.748	.859	.401	.564
DEQ Intensity P.M	Correlation	.149	-.253	.092	-0.213	-.259	.205	.173
	Sig.	.282	.065	.519	.125	.058	.145	.210

Appendix Q The Correlation coefficient values (r) and p values of the associations between ocular symptoms and ocular surface sensitivity, integrity and tear function in females (continuation). Red figures indicate significance level at $p < 0.05$ while blue figures indicate $0.05 < p < 0.25$

Ocular Symptoms		Corneal Sensitivity	Inferior Conjunctival Sensitivity	Tear Osmolarity	Tear Volume	NBUT	Corneal Staining	Conjunctival Staining
DEQ Bother	Correlation	.114	-.232	.137	-.251	-.132	.211	.143
	Sig.	.411	.091	.336	.070	.343	.133	.304
DEQ Total	Correlation	.132	-.250	.136	.265	-.265	.103	.143
	Sig.	.340	.068	.341	.210	.053	.469	.302
NRS Comfort	Correlation	-.148	.172	-.037	.227	.216	-.271	-.361**
	Sig.	.290	.219	.798	.106	.121	.055	.008
NRS Dryness	Correlation	-.206	-.073	.104	.085	.314*	-.149	-.335*
	Sig.	.136	.599	.468	.547	.021	.293	.013
NRS Foreign Body	Correlation	-.211	.029	.073	.263	.133	-.111	-.180
	Sig.	.129	.838	.614	.059	.342	.438	.198
NRS Burning	Correlation	-.154	-.037	.022	.274*	.178	-.033	-.182
	Sig.	.272	.792	.878	.049	.201	.818	.193
NRS Watery	Correlation	-.046	.054	-.151	-.132	.056	-.174	.058
	Sig.	.744	.700	.297	.351	.692	.221	.677

Appendix R The Correlation coefficient values (r) and p values of the associations between ocular symptoms and Meibomian gland assessments in all subjects. Red figures indicate significance level at p< 0.05 while blue figures indicate 0.05 <p < 0.25.

Ocular Symptoms		Marx's Line	Number of patent glands	Number of glands	Number of Capped glands	Meibomian gland Expressibility
OSDI	Correlation Coefficient	-.048	.176	-.032	-.151	-.057
	Sig. (2-tailed)	.684	.130	.800	.196	.627
OCI	Correlation Coefficient	.030	0.04	-0.054	-0.07	-0.021
	Sig. (2-tailed)	.795	0.726	0.648	0.546	0.866
SESOD	Correlation Coefficient	-.030	.173	.077	-.037	-.108
	Sig. (2-tailed)	.798	.136	.537	.749	.354
DEQ5	Correlation Coefficient	-.060	.028	-.108	-.112	-.073
	Sig. (2-tailed)	.608	.810	.386	.335	.534
DEQ Frequency	Correlation Coefficient	.039	.072	-.137	-.016	-.122
	Sig. (2-tailed)	.737	.537	.271	.891	.298
DEQ Intensity	Correlation Coefficient	.052	.089	-.085	.016	-.152
	Sig. (2-tailed)	.656	.442	.498	.889	.192
DEQ Intensity A.M	Correlation Coefficient	.050	.215	.016	-.127	-.033
	Sig. (2-tailed)	.668	.063	.900	.275	.778
DEQ Intensity P.M	Correlation Coefficient	-.011	.026	-.154	-.057	-.069
	Sig. (2-tailed)	.923	.824	.218	.625	.558

Appendix R **The Correlation coefficient values (r) and p values of the associations between ocular symptoms and Meibomian gland assessments in all subjects (continuation).** Red figures indicate significance level at p< 0.05 while blue figures indicate 0.05 <p < 0.25.

Ocular Symptoms		Marx's Line	Number of patent glands	Number of glands	Number of Capped glands	Meibomian gland Expressibility
DEQ Bother	Correlation Coefficient	.002	.112	-.137	-.097	-.078
	Sig. (2-tailed)	.987	.337	.274	.405	.505
DEQ Total	Correlation Coefficient	.034	.033	-.113	-.045	-.114
	Sig. (2-tailed)	.774	.776	.366	.701	.330
NRS Comfort	Correlation Coefficient	.119	-.123	.019	.045	.017
	Sig. (2-tailed)	.308	.293	.881	.703	.885
NRS Dryness	Correlation Coefficient	.095	-.331**	-.149	.097	.054
	Sig. (2-tailed)	.414	.004	.232	.403	.644
NRS Foreign Body	Correlation Coefficient	.015	-.224	.040	-.025	.181
	Sig. (2-tailed)	.898	.053	.749	.833	.122
NRS Burning	Correlation Coefficient	-.023	-.202	-.055	.021	.044
	Sig. (2-tailed)	.842	.082	.664	.857	.709
NRS Watery	Correlation Coefficient	-.007	-.138	.074	.069	-.004
	Sig. (2-tailed)	.952	.238	.558	.557	.971

Appendix S The Correlation coefficient values (r) and p values of the associations between ocular symptoms and Meibomian gland assessments in females. Red figures indicate significance level at p< 0.05 while blue figures indicate 0.05 <p < 0.25.

Ocular symptoms		Marx's Line	Number of patent gland	Number of glands	Number of Capped glands	Meibomian gland Expressibility
OSDI	Correlation Coefficient	-.041	.050	-.016	-.159	-.006
	Sig. (2-tailed)	.772	.722	.912	.255	.969
OCI	Correlation Coefficient	.030	-.037	.016	-.031	-.045
	Sig. (2-tailed)	.830	.789	.912	.823	.747
SESOD	Correlation Coefficient	.028	.173	.077	.044	-.075
	Sig. (2-tailed)	.841	.210	.604	.751	.595
DEQ5	Correlation Coefficient	-.046	-.098	-.126	-.026	.003
	Sig. (2-tailed)	.739	.483	.392	.852	.985
DEQ Frequency	Correlation Coefficient	.018	-.008	-.107	-.008	-.085
	Sig. (2-tailed)	.899	.956	.471	.954	.543
DEQ Intensity	Correlation Coefficient	.037	-.010	-.047	.006	-.129
	Sig. (2-tailed)	.788	.943	.751	.967	.359
DEQ Intensity A.M	Correlation Coefficient	.028	.128	.065	-.119	.040
	Sig. (2-tailed)	.841	.357	.660	.391	.774
DEQ Intensity P.M	Correlation Coefficient	.005	-.087	-.169	-.010	-.027
	Sig. (2-tailed)	.969	.532	.249	.942	.849
DEQ Bother	Correlation Coefficient	.002	-.036	-.120	-.066	-.019
	Sig. (2-tailed)	.987	.794	.415	.634	.894

Appendix S The Correlation coefficient values (r) and p values of the associations between ocular symptoms and Meibomian gland assessments in females (continuation). Red figures indicate significance level at p< 0.05 while blue figures indicate 0.05 <p < 0.25.

Ocular symptoms		Marx's Line	Number of patent gland	Number of glands	Number of Capped glands	Meibomian gland Expressibility
DEQ Total	Correlation Coefficient	-.009	-.003	-.098	-.001	-.083
	Sig. (2-tailed)	.951	.982	.509	.996	.555
NRS Comfort	Correlation Coefficient	.101	-.090	.005	-.058	-.062
	Sig. (2-tailed)	.470	.522	.972	.681	.664
NRS Dryness	Correlation Coefficient	.083	-.274*	-.162	.045	.035
	Sig. (2-tailed)	.552	.045	.270	.748	.802
NRS Foreign Body	Correlation Coefficient	-.022	-.196	.129	-.084	.228
	Sig. (2-tailed)	.875	.159	.388	.551	.103
NRS Burning	Correlation Coefficient	.034	-.182	-.062	.035	.118
	Sig. (2-tailed)	.809	.192	.680	.802	.404
NRS Watery	Correlation Coefficient	-.055	-.038	.088	-.004	-.048
	Sig. (2-tailed)	.695	.785	.557	.979	.735

Appendix T The Correlation coefficient values (r) and p values of the associations between sex hormones, ratio of oestradiol to androgen and study variables in PMW with dry eye (continuation). Red figures indicate significance level at p< 0.05 while blue figures indicate 0.05 <p < 0.25.

		Years Meno	vaso	psycho	physical	sexual	Osmo	PRT	Schm	NIBUT
E2	Correlation Coefficient	.147	-.148	.081	.135	-.232	.199	.034	.151	.004
	Sig. (2-tailed)	.336	.332	.598	.377	.124	.190	.824	.323	.981
DiolG	Correlation Coefficient	.250	-.139	.092	.185	-.019	.074	.093	-.050	.256
	Sig. (2-tailed)	.097	.361	.546	.223	.902	.630	.542	.743	.089
DHEAS	Correlation Coefficient	.000	.192	.243	.119	.117	.050	.210	-.211	-.253
	Sig. (2-tailed)	.998	.207	.108	.435	.444	.743	.166	.164	.093
E2: DG	Correlation Coefficient	-.109	-.081	.034	-.050	-.249	.186	-.078	.158	-.154
	Sig. (2-tailed)	.477	.595	.825	.746	.099	.222	.610	.300	.312

Appendix T The Correlation coefficient values (r) and p values of the associations between sex hormones, ratio of oestradiol to androgen and study variables in PMW with dry eye (continuation). Red figures indicate significance level at $p < 0.05$ while blue figures indicate $0.05 < p < 0.25$.

		CS	ICJS	CSt	ICJSt	Marx	Expressibility	NoCap	NoGland	Vascularity	Telangiectasia
E2	Correlation Coefficient	-.118	.014	.563**	.184	-.003	.095	.079	.237	.286	.297*
	Sig. (2-tailed)	.440	.928	.000	.226	.985	.536	.605	.117	.056	.047
DiolG	Correlation Coefficient	-.145	-.118	.184	-.074	.044	.238	-.104	.204	.149	.070
	Sig. (2-tailed)	.343	.439	.231	.629	.776	.115	.498	.180	.329	.649
DHEAS	Correlation Coefficient	-.055	.163	.281	-.126	.036	.126	-.059	.279	.202	-.012
	Sig. (2-tailed)	.722	.286	.064	.411	.816	.411	.701	.063	.183	.938
E2DG	Correlation Coefficient	-.034	.162	.324*	.257	-.082	-.007	.147	.069	.152	.246
	Sig. (2-tailed)	.825	.288	.032	.088	.590	.963	.334	.650	.320	.104

Appendix T The Correlation coefficient values (r) and p values of the associations between ocular symptoms and the other study variables in PMW with dry eye (continuation). Red figures indicate significance level at p< 0.05 while blue figures indicate 0.05 <p < 0.25.

		Years Meno	vasomotor	psycho	physical	sexual	Osmo	PRT	Schm	NBUT	Oestradiol	DiolG	DHEAS	E2DG
Years Meno	Correlation Coefficient	1.000	.043	.045	.236	.079	-.089	.010	.114	.238	.147	.250	.000	-.109
	Sig. (2-tailed)		.777	.767	.118	.608	.560	.950	.457	.115	.336	.097	.998	.477
OSDI	Correlation Coefficient	.095	-.071	.111	-.062	-.168	.170	-.003	-.012	.158	.097	.250	-.046	-.045
	Sig. (2-tailed)	.534	.642	.468	.687	.271	.264	.983	.935	.299	.524	.098	.763	.768
OCI	Correlation Coefficient	-.151	.174	.186	.017	.013	-.052	.042	.042	.011	-.121	-.049	-.067	-.026
	Sig. (2-tailed)	.322	.253	.220	.913	.930	.736	.784	.787	.941	.427	.748	.661	.866
OCI_Int	Correlation Coefficient	-.093	-.123	-.194	-.215	-.244	.126	.040	.066	.056	.014	-.074	-.076	.062
	Sig. (2-tailed)	.542	.420	.202	.156	.106	.411	.794	.668	.714	.930	.631	.621	.685
OCI_Freq	Correlation Coefficient	.007	.102	.022	-.103	-.009	-.074	-.063	-.025	-.059	-.196	-.151	.045	-.052
	Sig. (2-tailed)	.962	.503	.883	.501	.955	.628	.679	.871	.702	.197	.322	.769	.734

Appendix T The Correlation coefficient values (r) and p values of the associations between ocular symptoms and study variables in PMW with dry eye (continuation). Red figures indicate significance level at $p < 0.05$ while blue figures indicate $0.05 < p < 0.25$.

		CS	ICJS	CSt	ICJSt	Marx	Expressibility	NoCap	NoGland	Vascularity	Telangiectasia
Years Meno	Correlation Coefficient	.038	-.004	.095	.161	.044	.285	-.043	.162	.148	-.029
	Sig. (2-tailed)	.804	.977	.541	.290	.776	.058	.781	.286	.331	.850
OSDI	Correlation Coefficient	-.009	.042	-.130	-.171	-.020	.068	-.042	-.276	.044	.167
	Sig. (2-tailed)	.953	.783	.402	.260	.895	.655	.783	.067	.775	.274
OCI	Correlation Coefficient	.095	-.066	-.001	-.078	-.091	.102	.389**	-.131	-.298*	-.144
	Sig. (2-tailed)	.537	.665	.997	.611	.550	.504	.008	.391	.047	.346
OCI_Int	Correlation Coefficient	.008	-.118	.017	.049	.056	.289	.337*	.049	-.138	-.062
	Sig. (2-tailed)	.960	.439	.913	.747	.715	.054	.024	.749	.367	.685
OCI_Freq	Correlation Coefficient	.016	-.005	-.153	.020	-.004	.303*	.375*	-.018	-.237	-.162