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Itchy-Dry Eye Associated with Polycystic Ovary Syndrome

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ABSTRACT

BACKGROUND: We aimed to define the ocular symptomatology of women with polycystic ovaries and hyperandrogenism.

METHODS: Sixteen of 62 patients with polycystic ovaries (PCO) had hyperandrogenism (PCOS). Eight severely affected patients were placed on anti-androgen therapy. Women with a history of ocular symptoms (20/62 = 32.3%; 15/16 PCOS=93.7%; and 5/46 PCO=10.8%) received an eye examination with conjunctival impression cytology. Tear function (tear break-up time, Schirmer I test) was evaluated along with goblet cell number, immunostaining and RT-PCR for mucins MUC1 and MUC5AC. Western blot analysis of MUC5AC was carried out on tears of three patients before and after therapy.

RESULTS: Fifteen of 16 women (93.7%) with PCOS had ocular symptomatology: conjunctival hyperemia, follicular reactions, filamentous surface mucus, superficial punctate keratopathy, itching, dryness and burning. A high number of goblet cells was accompanied by a reduction of tear break-up time ($p<.05$). PCR analysis of conjunctival epithelium and Western blot analysis of tears demonstrated greater MUC5AC levels while its immunolocalization was reduced. Tear break-up time and symptomatology improved in all treated patients ($p<.05$). In three treated patients from whom tears were collected, MUC5AC was hugely increased at baseline. These findings normalized after 4 months of treatment. Similar ocular findings were observed in five of 46 patients (10.8%) without evidence of hyperandrogenism.

CONCLUSIONS: Females with PCOS were more likely to have itchy-dry eyes, increased goblet cell density and changes in the localization of MUC5AC. Evaluation of the ocular surface should be included in the differential diagnosis of PCOS.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine abnormality in women of reproductive age. With a prevalence estimated between 6.5 and 8%[1], 4 million women in the United States and approximately 105 million women worldwide are affected. Although the clinical, biochemical and diagnostic features of PCOS are well defined, there is no consensus as to the pathognomonic criteria for diagnosis of this multi-faceted disorder.[2] According to the Rotterdam definition, two of three criteria must be met to fit the definition of PCOS: chronic anovulation, clinical and/or biochemical evidence of hyperandrogenism and polycystic ovaries.[3] This condition has clinical consequences that extend beyond the reproductive system, including type2 diabetes, obesity and a predisposition to cardiovascular diseases and thrombosis.[4-9]

Sex hormones have been known for some time to influence the anatomical and functional structures of the ocular surface, particularly all components of the tear film: the lacrimal gland, the conjunctiva, goblet cells and their mucin products, the meibomian glands and the cornea.[10-12] Dysfunction of sex hormones has been linked to the severity, progression and treatment response of two major ocular conditions, dry eye[13] and vernal keratoconjunctivitis.[14] In particular, androgen deficiency in women results in meibomian gland dysfunction, tear film instability and dry eye in menopause[15], aging, autoimmune diseases[16] and premature ovarian failure.[17] In contrast, the physiologic rise of androgen serum levels at puberty is associated with improvement of signs and symptoms of vernal keratoconjunctivitis, an allergic eye disease of young boys in pre-puberty.[18] At present, it is not clear if high androgen levels induce changes to the ocular surface.

Young women with bilateral, persistent ocular surface discomfort characterized by itching, excessive mucus production, dryness and contact lens intolerance are a major challenge in our Cornea and External Diseases unit. Although these patients have signs and symptoms related to the major ocular surface

disorders, dry eye and ocular allergy, the anamnestic, clinical and diagnostic features do not fulfil the standard criteria. These patients share a common anamnestic finding: a previous and/or concomitant diagnosis of polycystic ovaries detected either by ultrasonography alone or with evidence of hyperandrogenism.

In the present report, we describe signs and symptoms related to ocular allergy and dry eye in approximately 94 percent of a series of young women with a diagnosis of PCOS. We propose that ocular surface symptoms are a distinct clinical entity associated with this common endocrinopathy.

METHODS

Study Protocol

This study was carried out with approval from the Institutional Review Board and the Intramural Committee. Written informed consent was obtained from all subjects.

From March 2005 to March 2006, we evaluated 62 consecutive females (age 25.4±5.27 years) with a diagnosis of polycystic ovaries detected by ultrasonography. All patients repeated the pelvic ultrasound examination and underwent clinical and biochemical evaluations to confirm the diagnosis of PCOS. In the early follicular phase (day 7-8) of the menstrual cycle, blood samples were collected from all women. Plasma levels of the following circulating hormones were investigated using radioimmunoassay (RIA), electrochemiluminescence immunoassay (ECLIA) or immunoradiometric assay (IRMA): total testosterone, free testosterone, 3-α-diologlucuronide, dihydrotestosterone (DHT), Δ-4-androstenedione, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEA-S), estrone, 17-β-estradiol, progesterone and sex hormone binding globulin (SHBG). Based on the clinical, ecographic and biochemical/hormonal parameters, women were diagnosed with: polycystic ovary syndrome (PCOS, N=16), having fullfilled all Rotterdam ESHRE/ASRM 2003 diagnostic criteria[19, 20] or

polycystic ovary (PCO, N=46) having ecographic but not clinical and/or hormonal evidence of hyperandrogenism.[21]

All subjects completed a questionnaire regarding the presence, onset and duration of ocular surface symptoms (dryness, itching, tearing, hyperemia, burning, foreign body sensation, photophobia and secretion/mucous discharge), previous and/or concomitant systemic or ocular diseases with special reference to allergy, use of contact lenses and history of any ocular or systemic treatment since their initial diagnosis of PCO. The age of onset and disease duration of PCO was also recorded.

Twenty patients (15 with PCOS; 5 with PCO) with ocular symptoms underwent a complete ophthalmologic examination, including visual acuity, biomicroscopy, intraocular pressure measurement and fundus oculi examination. For a complete differential diagnosis, a conjunctival swab was collected for identification of *Chlamydia* infection by immunohistochemistry. Skin prick tests and/or RAST were carried out to define the allergic status of each patient.

Tear functional tests (Schirmer I test, tear break-up time) were also performed in all 20 symptomatic PCO/PCOS patients and in 8 age- and sex-matched controls. The Schirmer I test without anesthesia was used to evaluate aqueous tear production: values greater than or equal to 10 mm of strip wetting in 5 minutes were considered normal and those less than 10 mm pathologic.[22] Tear film stability was evaluated using the fluorescein tear film break-up time (BUT): values greater than or equal to 10 seconds were considered normal and those less than 10 seconds pathologic.[22] Bulbar conjunctival impression cytology was also performed in all symptomatic women and in controls to evaluate goblet cell density by periodic acid Schiff (PAS) staining [23], and the conjunctival mucins MUC1 and MUC5AC by immunohistochemistry and RT-PCR.

Eight patients with overt hyperandrogenism (Ferriman & Gallwey score >15) and complaining of severe hyperseborrhea and hirsutism were placed on systemic anti-androgenic therapy with sprinolactone 100-150 mg/day and estroprogestins. A follow-up clinical examination was carried out 4

months after onset of therapy. Tear samples were collected from 3 of these patients at baseline and at the 4-month follow-up visit and Western blot analysis of MUC5AC was performed.

Procedures

Conjunctival epithelium samples were obtained from all 20 PCO/PCOS patients with ocular symptomatology and from 8 age/sex-matched controls. In particular, four conjunctival impression cytology samples (temporal, nasal, inferior and superior bulbar conjunctiva) were obtained from each eye using 0,4 μm Millicel sterile membranes (Millipore Corp. Billerica, MA, USA). Periodic acid Schiff staining (PAS; Bio-Optica, Milan, Italy) was carried out on prefixed (Microscopy Merckofix spray fixative, Merck KgaA, Darmstadt, Germany) samples. Quantification of goblet cells was carried out at 40X magnification in a masked fashion (E400 light microscope; Nikon, Tokyo, Japan). Data are expressed as mean ± SD of 3 consecutive optic fields for each impression cytology sample.

RT-PCR was performed to assess MUC1 and MUC5AC mRNA expression in impression cytology samples. Total RNA was extracted according to the Puregene RNA isolation kit (Gentra systems, Minneapolis, MN, USA). Normalized total RNA (1μg) was reverse-transcripted (MML-V, Finnzyme, Celbio) and cDNAs were amplified according to the standardized SYBR Green PCR procedure (Applied Biosystems, Foster City, CA). Ct values from specific targets (MUC1, MUC5AC) or the standard (GAP) amplification were entered into the REST© software. Results are expressed as gene upregulation or downregulation fold ratio.

MUC1 and MUC5AC immunostaining was carried out on impression cytology samples using specific anti-human primary monoclonal antibodies (10μg/mL; Oxford Biotechnology Limited ltd., UK) and developed according to the avidin-biotin complex/peroxidase technique (Vectastain *Elite*ABC kit;

Vector Laboratories, Inc., Burlingame, CA, USA). As a negative control, samples were stained with a non-specific isotype antibody (Vector). Positive cells were counted at 40X magnification in a masked fashion (E400 light microscope, Nikon). Data are expressed as mean ± SD of 3 consecutive optic fields for each quadrant used (superior quadrant for MUC1 and nasal quadrant for MUC5AC).

Western Blot analysis for MUC5AC was carried out on tear samples of 3 patients before and 4 months after systemic anti-androgenic therapy. Tears were collected by microsponges and immersed in 50μL of lysis buffer containing 10 μg/mL aprotinin and 1 mM PMSF (T-PER, Tissue Protein Extractor, Pierce, IL, USA). Equivalent amounts of protein (20 μg/line), quantified by Bio-Rad assay (Bio-Rad Laboratories, Inc, Hercules, CA, USA), were subjected to 10% SDS-PAGE electrophoresis, blotted onto Hy-bond membranes (Bio-Rad), probed and developed according to the ECL technique (SuperSignal West Pico Trial, Pierce). Bands were acquired in a Kodak Imager Station (Kodak 550, Eastman Kodak Company Sci. Imaging Systems, Rochester, NY), densitometrically evaluated and the images processed by Adobe Photoshop 7.0 (Adobe Systems Inc., San Jose, CA, USA). Data are expressed as mean ± SD optical density (OD) of normalized samples, according to the densitometric analysis.

Statistical Analysis

ANOVA-Tukey/Kramer post-hoc analysis was carried out to compare the differences between groups (STATVIEW II for PC; Abacus Concepts. Inc., Berkeley, CA, USA).[24] Data are expressed as mean ± SD and a $p \leq .05$ was considered statistically significant.

RESULTS

Sixteen of 62 patients were diagnosed with PCOS according to the Rotterdam 2003 criteria, while the remaining 46 patients were diagnosed with ecographic evidence of polycystic ovary (PCO) only without clinical/biochemical evidence of hyperandrogenism.

Twenty of 62 (32.3%) PCO/PCOS patients presented with moderate to severe ocular signs and symptoms. Fifteen of the 16 women with PCOS (93.7%) and 5 of the 46 PCO patients (10.8%) complained of the presence of bilateral ocular symptoms. These symptoms were manifested approximately 3 years (mean 38±23 months) after the initial ecographic diagnosis of polycystic ovaries. Itching, dryness, foreign body sensation, photophobia and burning were the most frequently cited symptoms. Six patients also presented with superficial punctate keratitis at the slit lamp examination (Table 1). Ocular symptoms were persistent despite the use of tear substitutes and/or ophthalmic antiallergic, mucolytic or vasoconstrictor eyedrops.

Slit lamp examination revealed hyperemia and mild follicular and papillary reactions of both the upper and lower tarsal conjunctiva. In 4 patients, excessive mucus production was observed, with long filaments extending on the ocular surface (Figure 1). Nine of 10 (8 PCOS, 2 PCO) contact lens wearers (90%) discontinued lens wear for complete intolerance due to foreign body sensation and dryness. One PCO patient had a positive *Chlamydia* swab with complete remission of symptoms following specific therapy while 4 allergic, skin-test positive patients did not benefit from ocular anti-allergic therapy.

Tear function tests in symptomatic patients demonstrated that the Schirmer I test was in the normal range (13.7±3.4 mm/5min) in 94% of patients, while tear film break-up time was significantly reduced ($p<.05$) compared to healthy age/sex-matched controls (4.5±1.5 sec vs 9.1±1.2 sec, respectively). No significant difference was found between PCOS and PCO patients (Figure 2A).

PAS staining of conjunctival impression cytology samples from PCOS women showed a significant increase in goblet cell number compared to controls (Figure 3A-B), with a mean value of

80±37/mm^2 vs. 20±4/mm^2 ($p< .05$). While conjunctival immunolocalization of the secretory mucin MUC5AC was significantly reduced (33±13% vs. 99±1%; $p< .05$) in PCOS versus healthy samples in terms of positive goblet cell number (Figure 3C-D), immunolocalization of membrane-associated MUC1 was not altered. Furthermore, conjunctival mRNA expression of MUC5AC was markedly increased (15.8-fold increase, $p<.05$), while that of MUC1 was only slightly increased (1.8-fold increase, $p>.05$) in PCOS patients compared to controls (Figure 4A). The specificity of this PCR analysis was confirmed by the single melting curve evaluation (Figure 4B-C). Western blot analysis of tears collected from three PCOS and three healthy subjects revealed a 177% increase of the secretory mucin MUC5AC in PCOS samples (36±14 O.D. vs. 13±3 O.D.; $p>.05$).

In the 8 PCOS patients treated for four months with systemic anti-androgenic therapy consisting of spironolactone and estroprogestins as well as local tear substitute therapy, the ocular symptoms and tear film break-up time (6.6±1.5 sec, $p< .05$) significantly improved (Figure 2B). In addition, in the three treated patients from whom tears were collected, a 47% decrease in tear MUC5AC was observed when compared to baseline (19±3 vs. 36±14, $p>.05$). In the 4 treated contact lens wearers, intolerance was reduced.

DISCUSSION

We describe the presence of conjunctival hyperemia, mild follicular and papillary reactions, mucus hypersecretion and occasional superficial punctate keratopathy associated with moderate to severe itching, dryness, burning, and foreign body sensation in 94% of young women with polycystic ovary syndrome (PCOS). In these patients, ocular signs and symptoms were manifested approximately 3 years after the first ultrasonographic finding of polycystic ovaries.

These signs and symptoms could be ascribed to two distinct entities, ocular allergy and dry eye, yet clinical features did not fulfil standard criteria used for the diagnosis of these common external eye diseases. In fact, there was no evidence of systemic sensitization, no history or evidence of previous ocular allergy, no association with other allergic diseases and no response to common anti-allergic treatment to support the diagnosis of allergic conjunctivitis. Similarly, normal values of the Schirmer test, negative vital staining of the ocular surface, the prevalence of itching as a symptom and the atypical age of these patients did not correspond to a clinical diagnosis of dry eye.[25] Toda and colleagues observed a similar phenomenon,[26] describing the occurrence of dry eye defined only by decreased tear break-up time occasionally associated with allergic conjunctivitis. They concluded that decreased break-up time was due to decreased goblet cell density and that this was a component of the ocular allergic pathology. We suspect that the unusual coupling of itching and dryness is a distinct clinical entity associated with hormonal imbalance and we have designated this condition the *Itchy-Dry Eye Associated* (IDEA) *Syndrome*.

Mucus hypersecretion was a constant finding in our patients. The presence of mucus hypersecretion and long filaments of mucus on the ocular surface was probably responsible for the contact lens intolerance noted in 90% of wearers. This condition is similar to a previously described entity termed ‘mucus fishing syndrome’ whose pathogenesis is still poorly understood. McCulley and co-workers described the presence of long threads of mucus on the ocular surface as a response to non-specific damage from underlying diseases including keratoconjunctivitis sicca, blepharitis and ocular allergy.[27] We suspect that hormonal abnormalities might lead to these ocular repercussions far from the usually suspected target organs.

Although the underlying mechanism of itchy-dry eye has not been clarified, there is clear evidence that the ocular surface is modulated by sex hormones such as estrogens, progesterone and androgens. Specific receptors for these hormones have been found on the healthy human conjunctiva[28]

and in dry eye[13] and are over-expressed in vernal keratoconjunctivitis.[14] Androgens influence the lipid layer of the tear film by modulating meibomian gland secretion[29] and the aqueous layer of the tear film by modulating the lacrimal gland.[30] It is possible that, as described in other tissues, androgens may also control the mucous layer of the tear film by modulating gene expression of mucins,[31] high molecular weight glycoprotein components of mucus that protect and lubricate the ocular surface[32, 33] and the epithelial surface of the respiratory, gastrointestinal and reproductive tracts.[34,35] It is also possible that other factors, such as insulin resistance, are involved in the pathogenesis of itchy-dry eye: in fact PCOS patients typically have decreased insulin sensitivity and androgen activity may be abnormal in diabetic situations.[36]

The goblet cell hyperplasia observed in these patients is a common feature of ocular allergic diseases and asthma,[37] leading clinically to a hypersecretion of mucus. Results for the mucin MUC5AC were decidedly more complex. Compared to healthy subjects, significantly lower conjunctival tissue localization was observed coupled with a much greater conjunctival mRNA expression of MUC5AC. In the three patients from whom tears were collected, these findings were accompanied by a huge increase (177%) in tear levels of MUC5AC. The anomalous change in location from conjunctival tissue in normals to tears in PCOS patients appears to be pathogenic and may give rise to the origin of the abnormal mucous filaments accumulated on the ocular surface. A similar finding was observed in asthmatic patients, in whom MUC levels were inversely proportional; i.e., higher MUC levels in the epithelium were associated with lower levels of secreted MUC in induced sputum, and vice versa.[37]

In our study, women with PCOS were more likely to develop ocular signs and symptoms than patients with polycystic ovaries not accompanied by hyperandrogenism. The significant improvement of tear film break-up time and ocular symptoms in patients who received systemic antiandrogenic therapy indicate that hormonal imbalance is directly related to ocular manifestations and that evaluation of

ocular signs and symptoms could be an additional criterion for the differential diagnosis of PCOS from PCO.

In conclusion, itchy-dry eye symptomatology in polycystic ovary syndrome appears to be a distinct clinical entity that shares many characteristics with dry eye and ocular allergic disease but has the unique feature of a concomitant endocrinopathy. The finding that systemic anti-androgenic therapy improved ocular symptoms and the quality of tears confirms the premise that sex hormones modulate ocular surface and tear film health.

REFERENCES

1. Azziz R, Marin C, Hoq L, Badamgarav E, Song P. Health care-related economic burden of the polycystic ovary syndrome during the reproductive life span. J Clin Endocrinol Metab 2005;90(8):4650-8.

2. Azziz R. Diagnostic criteria for polycystic ovary syndrome: a reappraisal. Fertil Steril 2005;83(5):1343-6.

3. Franks S, McCarthy MI, Hardy K. Development of polycystic ovary syndrome: involvement of genetic and environmental factors. Int J Androl 2006;29(1):278-85; discussion 86-90.

4. Salzmann J, Jagger J. Central retinal vein occlusion associated with the polycystic ovarian syndrome. Int J Clin Pract 1997;51(5):339-41.

5. Zilliacus H, Nystroem B. Visual Disturbance, a Symptom in Stein-Leventhal's Syndrome. Acta Obstet Gynecol Scand 1964;42:SUPPL 6:161-5.

6. Balen A, Rajkowha M. Polycystic ovary syndrome--a systemic disorder? Best Pract Res Clin Obstet Gynaecol 2003;17(2):263-74.

7. Alexandraki K, Protogerou AD, Papaioannou TG, et al. Early microvascular and macrovascular dysfunction is not accompanied by structural arterial injury in polycystic ovary syndrome. Hormones (Athens) 2006;5(2):126-36.

8. Ehrmann DA, Liljenquist DR, Kasza K, Azziz R, Legro RS, Ghazzi MN. Prevalence and predictors of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2006;91(1):48-53.

9. Lakhani K, Leonard A, Seifalian AM, Hardiman P. Microvascular dysfunction in women with polycystic ovary syndrome. Hum Reprod 2005;20(11):3219-24.

10. Azzarolo AM, Eihausen H, Schechter J. Estrogen prevention of lacrimal gland cell death and lymphocytic infiltration. Exp Eye Res 2003;77(3):347-54.

11. Gao J, Lambert RW, Wickham LA, Banting G, Sullivan DA. Androgen control of secretory component mRNA levels in the rat lacrimal gland. J Steroid Biochem Mol Biol 1995;52(3):239-49.

12. Gollub EG, Waksman H, Goswami S, Marom Z. Mucin genes are regulated by estrogen and dexamethasone. Biochem Biophys Res Commun 1995;217(3):1006-14.

13. Sullivan DA, Sullivan BD, Evans JE, et al. Androgen deficiency, Meibomian gland dysfunction, and evaporative dry eye. Ann N Y Acad Sci 2002;966:211-22.

14. Bonini S, Lambiase A, Schiavone M, Centofanti M, Palma LA. Estrogen and progesterone receptors in vernal keratoconjunctivitis. Ophthalmology 1995;102(9):1374-9.

15. Mathers WD, Stovall D, Lane JA, Zimmerman MB, Johnson S. Menopause and tear function: the influence of prolactin and sex hormones on human tear production. Cornea 1998;17(4):353-8.

16. Sullivan DA, Wickham LA, Rocha EM, et al. Androgens and dry eye in Sjogren's syndrome. Ann N Y Acad Sci 1999;876:312-24.

17. Smith JA, Vitale S, Reed GF, et al. Dry eye signs and symptoms in women with premature ovarian failure. Arch Ophthalmol 2004;122(2):151-6.

18. Bonini S, Coassin M, Aronni S, Lambiase A. Vernal keratoconjunctivitis. Eye 2004;18(4):345-51.

19. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19(1):41-7.

20. Ehrmann DA. Polycystic ovary syndrome. N Engl J Med 2005;352(12):1223-36.

21. Battaglia C, Mancini F, Persico N, Zaccaria V, de Aloysio D. Ultrasound evaluation of PCO, PCOS and OHSS. Reprod Biomed Online 2004;9(6):614-9.

22. Holland. Ocular Surface Disease. Medical and Surgical Management: Springer; 2001.

23. Kinoshita S, Kiorpes TC, Friend J, Thoft RA. Goblet cell density in ocular surface disease. A better indicator than tear mucin. Arch Ophthalmol 1983;101(8):1284-7.

24. Wilcox. New statistical procedures for the social sciences: Modern Solution to Basic Problems. In. Hillsdale, NJ: Erlbaum; 1987.

25. Fujishima H, Toda I, Shimazaki J, Tsubota K. Allergic conjunctivitis and dry eye. Br J Ophthalmol 1996;80(11):994-7.

26. Toda I, Shimazaki J, Tsubota K. Dry eye with only decreased tear break-up time is sometimes associated with allergic conjunctivitis. Ophthalmology 1995;102(2):302-9.

27. McCulley JP, Moore MB, Matoba AY. Mucus fishing syndrome. Ophthalmology 1985;92(9):1262-5.

28. Wickham LA, Gao J, Toda I, Rocha EM, Ono M, Sullivan DA. Identification of androgen, estrogen and progesterone receptor mRNAs in the eye. Acta Ophthalmol Scand 2000;78(2):146-53.

29. Sullivan DA, Sullivan BD, Ullman MD, et al. Androgen influence on the meibomian gland. Invest Ophthalmol Vis Sci 2000;41(12):3732-42.

30. Azzarolo AM, Wood RL, Mircheff AK, et al. Androgen influence on lacrimal gland apoptosis, necrosis, and lymphocytic infiltration. Invest Ophthalmol Vis Sci 1999;40(3):592-602.

31. Mitchell S, Abel P, Madaan S, et al. Androgen-dependent regulation of human MUC1 mucin expression. Neoplasia 2002;4(1):9-18.

32. Gipson IK, Argueso P. Role of mucins in the function of the corneal and conjunctival epithelia. Int Rev Cytol 2003;231:1-49.

33. Inatomi T, Spurr-Michaud S, Tisdale AS, Gipson IK. Human corneal and conjunctival epithelia express MUC1 mucin. Invest Ophthalmol Vis Sci 1995;36(9):1818-27.

34. Hebbar V, Damera G, Sachdev GP. Differential expression of MUC genes in endometrial and cervical tissues and tumors. BMC Cancer 2005;5:124.

35. Rose MC. Mucins: structure, function, and role in pulmonary diseases. Am J Physiol 1992;263(4 Pt 1):L413-29.

36. Toda I, Sullivan BD, Wickham LA, Sullivan DA. Gender- and androgen-related influence on the expression of proto-oncogene and apoptotic factors mRNAs in lacrimal glands of autoimmune and non-autoimmune mice. J Steroid Biochem Mol Biol 1999; 71(1-2):49-61.

37. Fahy JV. Goblet cell and mucin gene abnormalities in asthma. Chest 2002;122(6 Suppl):320S-6S.

FIGURE LEGENDS

Figure 1. Slit lamp examination of ocular surface

Patients with PCOS and ocular symptomatology (N=20) presented with (asterisk) conjunctival hyperemia (A) and follicular reactions (B). In 23.53% of patients, mucous strands (arrows) were identified on corneal epithelium (C) and on conjunctival epithelium by Rose Bengal staining (D).

Figure 2. Fluorescein tear film break up time (BUT)

Symptomatic patients with PCOS (N=30 eyes) and PCO (N=10 eyes) showed a significant decrease in BUT (A) as compared to healthy age-matched controls (N=16 eyes). As shown in panel B, after four months of systemic therapy with spironolactone and estroprogestins, BUT significantly improved in PCOS patients (N=16 eyes). Values expressed as mean ± SEM.

Figure 3. Goblet cell and MUC5AC protein expression in conjunctival impression cytology samples

PAS-stained conjunctival impression cytology of one representative PCOS patient (A) clearly indicated a significant increase in the number of goblet cells compared to an age/sex-matched healthy control (B). Immunohistochemistry of conjunctival impression cytology samples showed a reduction in specific MUC5AC staining in goblet cells (arrows) in one representative PCOS patient (C) compared to an age/sex-matched healthy control (D). Magnification x400.

Figure 4. MUC1 and MUC5AC mRNA expression in the conjunctiva

Total RNA extraction, cDNA reverse transcription and specific amplification for MUC1 (for: 5'- TTC CCA GCC ACC ACT CTG ATA C -3' rev: 5'- AGT GCT GTG ATT GGA GGA GGT G-3'; 116 bps; J05582), MUC5AC (for: 5'- TCC ACC ATA TAC CGC CAC AGA -3' rev: 5'- TGG ACC GAC AGT

CAC TGT CAA C-3'; 103bps; AF015521) and GAPDH (for: 5'-GAA GGG GTC ATT GAT GGC AAC-3' rev: 5'-GGG AAG GTG AAG GTC GGA GTC-3'; 100bps; BC013310) primers were carried out on impression cytology samples. A significant increase in MUC5AC mRNA expression was detected in patients with PCOS (A). Panels B and C depict the specific melting curve of both set primers, confirming the specificity of the analysis.

Table 1. Characteristics of symptomatic patients

	N°	Age	Oligo-Amennorrhea Age at onset	Ovaries at Pelvic Ultrasonography	Hormonal Alterations *	Clinical signs of hyperandrogenemia	Major ocular signs	Major ocular symptoms
PCOS	1	24	21	↑Ovarian size Multiple cysts	↑Testosterone ↓SHBG	Acne, Hirsutism, Central Obesity	Hyperemia, Mucous Secretion, Punctate Keratitis	Foreign Body Sensation, Contact Lens Intolerance
	2	22	19	↑Ovarian size Multiple cysts	↑Estrone ↑Testosterone ↓SHBG	Acne	Hyperemia	Itching, Dryness
	3	26	26	Multiple cysts	↑Testosterone	Acne	Hyperemia, Mucous Secretion, Punctate Keratitis	Itching, Contact Lens Intolerance
	4	25	19	Multiple cysts	↑ Testosterone ↑DHEA-S ↑DHT	Acne	Hyperemia	Itching, Dryness Contact Lens Intolerance
	5	21	16	Multiple cysts	↑Testosterone	Acne	Hyperemia, Follicular reaction	Itching, Dryness
	6	21	19	↑Ovarian size Multiple cysts	↑Testosterone ↓SHBG	Acne, Hirsutism	Hyperemia	Itching, Dryness
	7	31	19	↑Ovarian size Multiple cysts	↑Estrone	Acne, Hirsutism	Hyperemia	Burning, Contact Lens Intolerance
	8	22	18	↑Ovarian size Multiple cysts	↑Testosterone ↓SHBG	Acne, Hirsutism, Androgenic Alopecia, Central Obesity	Hyperemia, Follicular reaction, Punctate Keratitis	Itching, Dryness, Contact Lens Intolerance
	9	28	17	↑Ovarian size Multiple cysts	↑Testosterone ↑ DHEA-S	Acne, Hirsutism	Hyperemia, Punctate Keratitis	Itching, Dryness, Photophobia, Foreign Body Sensation

	10	36	29	↑Ovarian size Multiple cysts	↑Testosterone ↑DHEA-S	Hirsutism, Central Obesity	Hyperemia	Dryness
	11	28	23	Multiple cysts	↑Testosterone	Acne	Hyperemia	Dryness Contact Lens Intolerance
	12	30	25	Multiple cysts	↑Estrone ↑DHEA-S	Hirsutism	Hyperemia, Mucous Secretion, Punctate Keratitis	Itching, Dryness, Foreign Body Sensation, Contact Lens Intolerance
	13	41	29	Multiple cysts	↑Testosterone	Acne	Hyperemia	Dryness, Photophobia
	14	28	28	Multiple cysts	↑Estrone	Acne	Hyperemia, Follicular reaction	Dryness Contact Lens Intolerance
	15	32	18	↑Ovarian size Multiple cysts	↑Δ4Androstenedione	Acne, Hirsutism	Hyperemia, Mucous Secretion, Follicular reaction, Punctate Keratitis	Itching, Dryness, Foreign Body Sensation
PCO	16	30	-	Micro-polycistic Ovaries	None	None	Hyperemia	Burning Contact Lens Intolerance
	17	24	-	Multiple cysts	None	None	Hyperemia, Follicular reaction	Itching, Dryness, Photophobia, Foreign Body Sensation
	18	26	-	Multiple cysts	None	None	Hyperemia	Photophobia
	19	23	-	Multiple cysts	None	None	Hyperemia, Follicular reaction	Itching, Dryness
	20	28	-	Multiple cysts	None	None	Hyperemia	Burning, Photophobia

*** Normal Ranges:** Testosterone 0.06-0.82 ng/ml (E.C.L.I.A.); Free Testosterone 0-5 pg/ml (R.I.A.); 3-α-Diologlucuronide 0.17-5 ng/ml (R.I.A.); DHT 35-160 pg/ml (R.I.A.); Δ-4-Androstenedione 1-2.5 ng/ml (R.I.A.); DHEA 1.5-9 ng/ml (R.I.A.); DHEA-S 1.8-7.7 μmol/l (E.C.L.I.A.); Estrone 40-110 pg/ml (R.I.A.); 17-β-Estradiol 12.5-166 (E.C.L.I.A.); Progesterone 0.2-1.5 ng/ml (E.C.L.I.A.); SHBG 20-90 nmol/l (I.R.M.A.).

Figure 1.

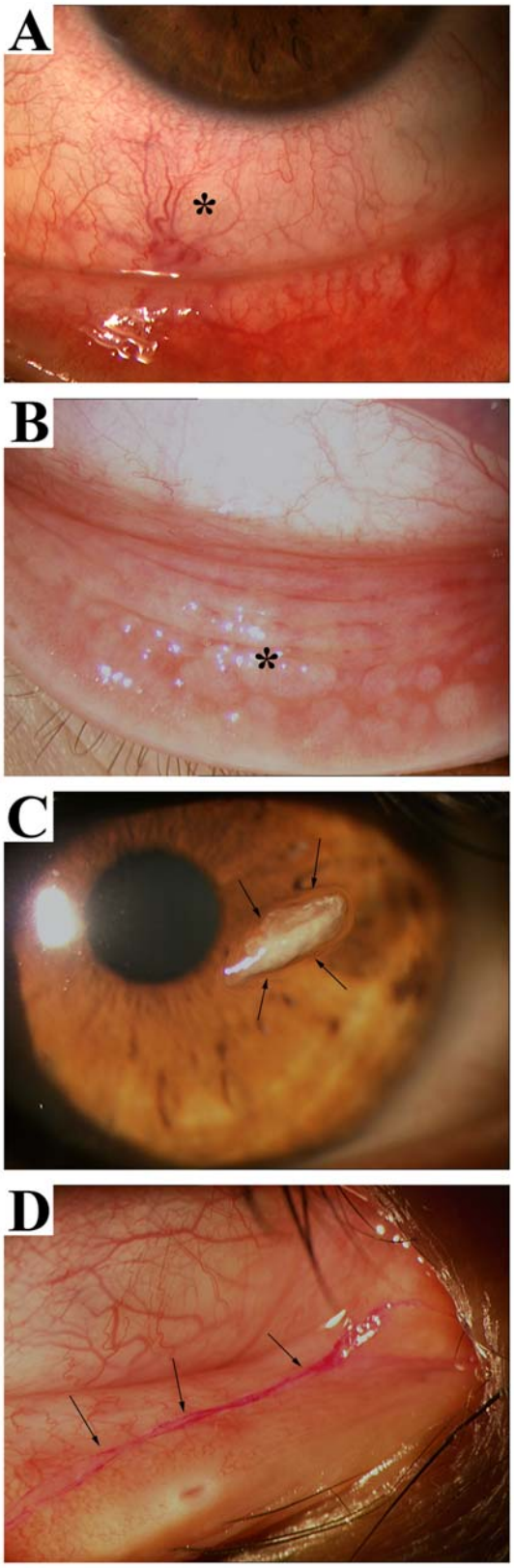


Figure 2.

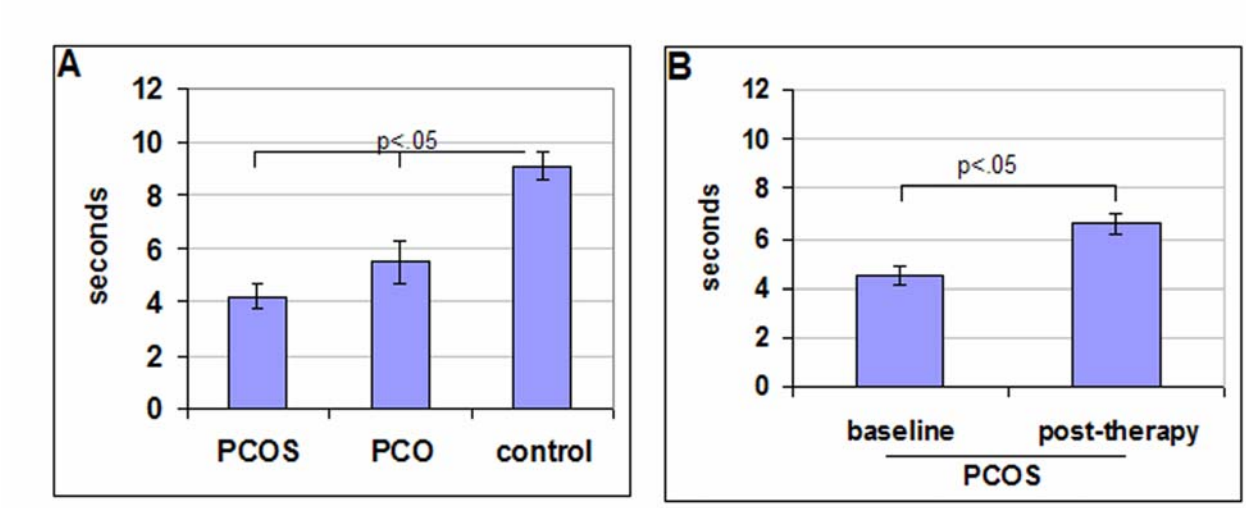

A
12
10
8
6
4
2
0
seconds
p<.05
PCOS
PCO
control
B
12
10
8
6
4
2
0
seconds
p<.05
baseline
post-therapy
PCOS

Figure 3.

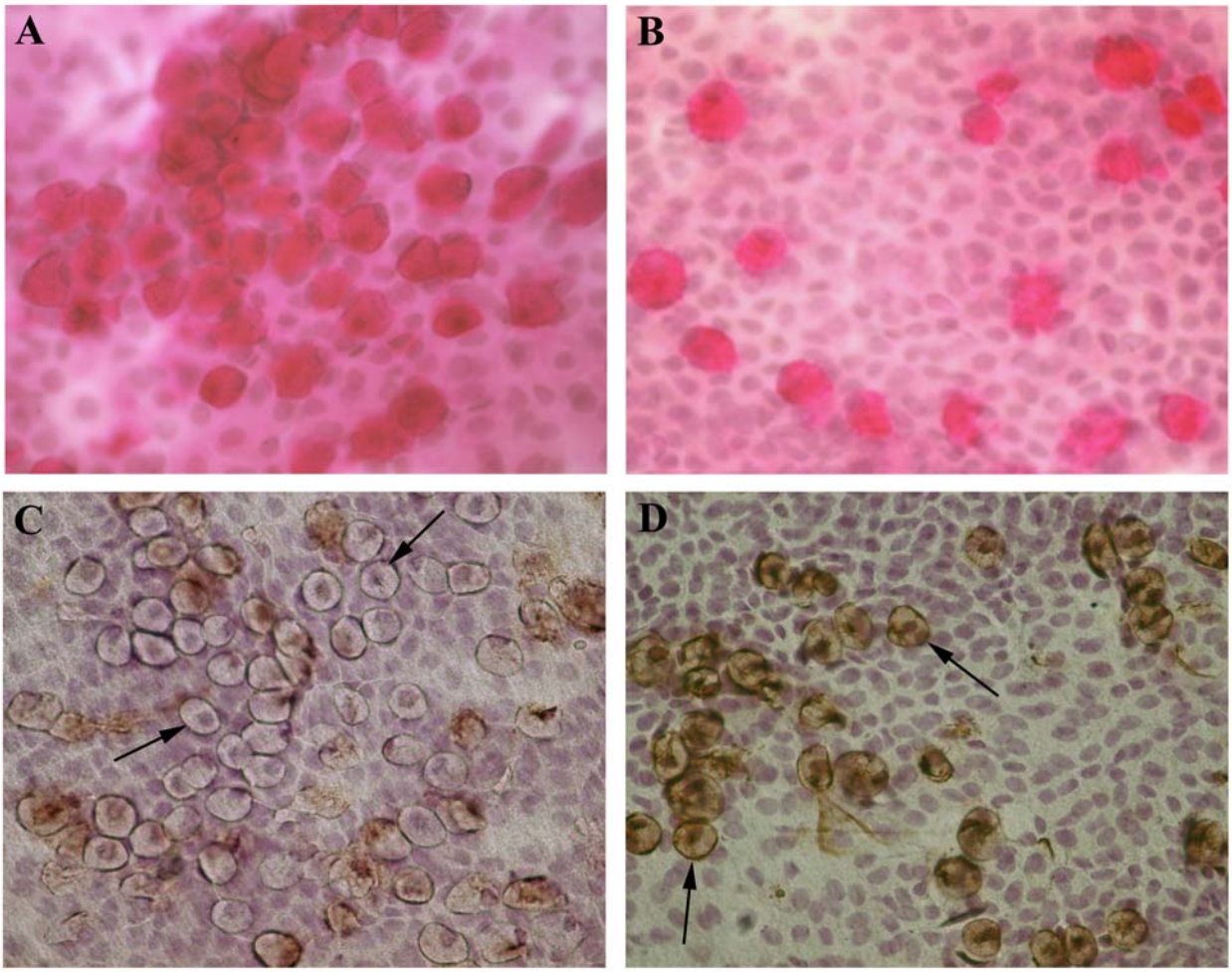
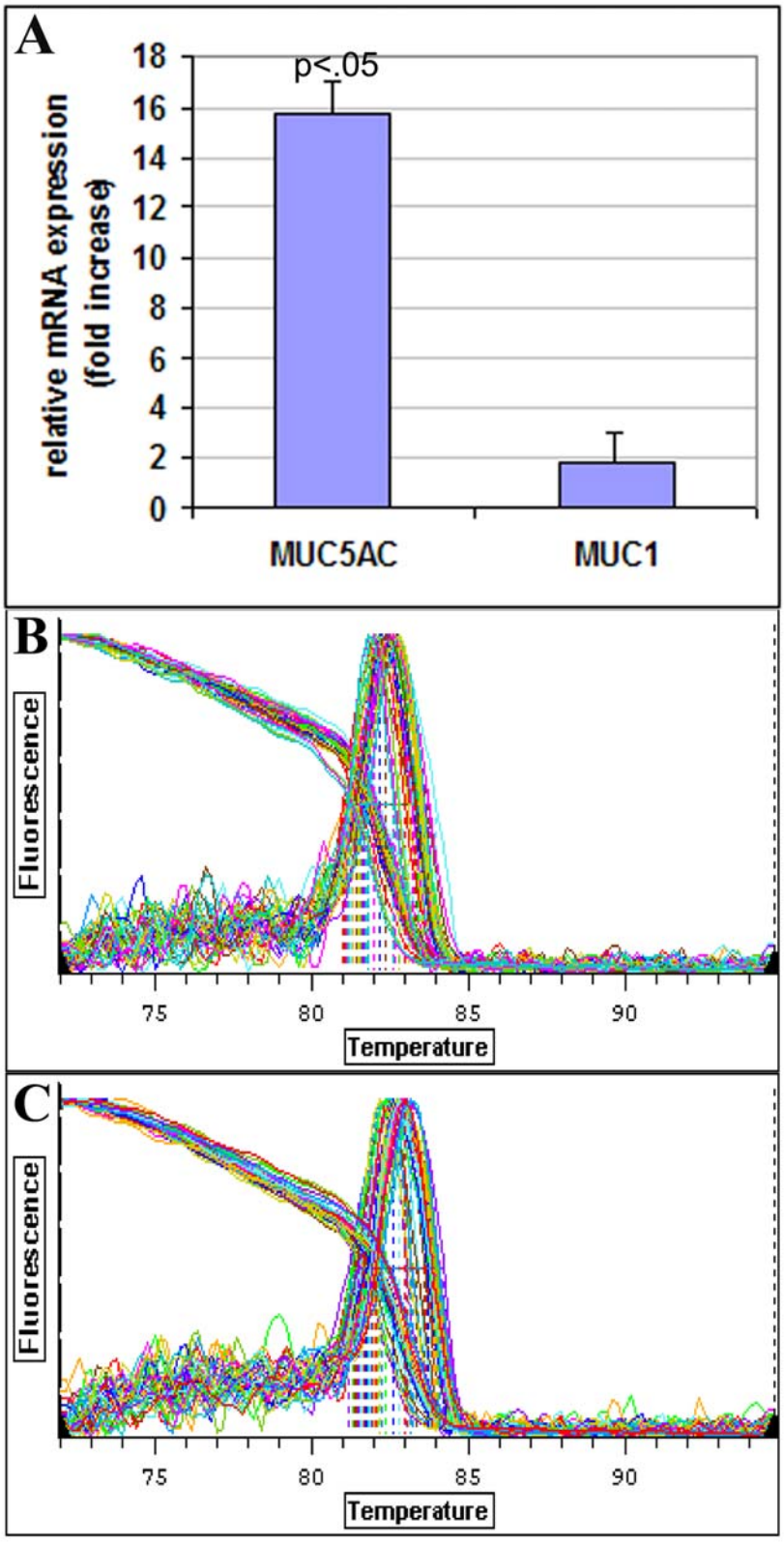


Figure 4.


A
p<.05
relative mRNA expression
(fold increase)
18
16
14
12
10
8
6
4
2
0
MUC5AC
MUC1
B
Fluorescence
75
80
85
90
Temperature
C
Fluorescence
75
80
85
90
Temperature