Title: The effects of a new generation oral contraceptive pill on the vocal folds of professional voice users: a pilot investigation

FILIPA M. B. LÃ, DAVID M. HOWARD, WILLIAM LEDGER, JANE W. DAVIDSON & GEORGINA JONES

The effects of a new generation oral contraceptive pill on the vocal folds of professional voice users: a pilot investigation

Logopedics Phoniatrics Vocology

Abstract

Few studies have been concerned with the effects of combined oral contraceptive pills (COCP) on those whose success depends on voice quality. A cohort study involving 20 female professional voice users (mean age = 26.60; SD = 6.319; range = 19-40 years) was carried out assessing the pattern of vibration of the vocal folds as a measure of irregularity of vocal fold vibration, based on (a) period-to-period frequency peaks (CFx) and (b) period-to-period amplitude peaks (CAx) in the cycle to cycle excitation of the vocal tract. These electrolaryngographic vocal measures and blood samples were collected at three specific points of the menstrual cycle, for both natural and COCP cycles. No significant differences were found on vocal parameters assessed between the natural and the combined oral contraceptive cycles. The anti-androgenic and anti-mineralocorticoid properties of this COCP were not found to have a systematic effect on the vocal parameters studied.

Key words: Professional voice; Sex steroid hormones; Combined oral contraceptive pill.

Introduction

The combined oral contraceptive pill (COCP), a hormone related medication containing both synthetic oestrogen and progesterone, is one of the most widely used contraceptive method.[1] However, its effects on the voice are still not clear, particularly for those who use their voices as a tool of trend (e.g. singers of different music genres, and teachers).

Previous studies investigating the effects of COCPs on the voice of professional classical singers have shown controversial results. On the one hand, there are studies suggesting direct and indirect negative effects on the voice associated with COCP use; however, recent studies could not find a negative effect of COCP use on the voice. In fact, the results of some of these studies suggested that COCP use might have a positive effect on voice quality through vocal stabilisation throughout the different phases of the menstrual cycle.

Earlier studies have shown that the use of old preparations containing 19-norsteroid derivates as progestogens lead to vocal virilisation, presenting symptoms such as vocal breaks, lowering of speaking vocal pitch, and hoarseness. These negative effects have been associated with the androgenic derivation of the progestogen component in these prepartions.[2-3] COCP preparations have been also associated with variations in voice quality by causing symptoms which indirectly interfere with voice production. For example, studies investigating the effects of high concentrations of synthetic oestrogens on the nasal mucosa suggested that changes in the squamous epithelium, oedema of the corium, glandular hyperplasia, cellular infiltration, and vascular changes were observed. [4] The authors hypothesised that the increase in hormonal concentrations of oestrogens were responsible for water retention and oedema of the connective tissue, supporting the idea that chronic inflammatory processes in the connective layer of several mucosal membranes of the body, including the vocal fold's mucosa, can be initiated by higher concentrations of oestrogens.[4]

Other symptoms which could indirectly interfere with vocal performance, such as "plugged up ears", vertigo, hearing difficulties, nasal congestion, and headache were also associated with the use of old high dosage COCP preparations.[5]

On the other hand, newer low dose COCP preparations containing newer progestogens, have not been associated with vocal problems. For example, in a study investigating the perceptual and acoustical effects (such as vocal ranges and fundamental frequency) of new COCPs on the voice of classically trained singers, no negative symptoms associated with COCP use could be found. The authors believe that with the pharmacological evolution of newer COCPs, side vocal effects have been reduced. However, monitorisation of the professional voice should be constantly performed during COCP use.[6] Other studies found that the use of certain COCPs might improve female voice quality during the menstrual cycle, bringing benefits for singers. When singers who had a history of irregular menstrual cycles and vocal problems were using a COCP, their body temperature and the speaking fundamental frequency was stabilised and raised to a higher pitch. Therefore, COCP use might help singers who have serious hormonal imbalances to stabilise their voices.[7] Finally, the results of the first double blind randomised placebo controlled trial assessing the effects of a low dose COCP containing drospirenone on the classically singing voice suggested that the use of this COCP seemed to contribute to the optimisation of the singers' performances.[8] The authors suggested that the effects of hormonal variations during the menstrual cycle, such as water retention in the vocal folds' mucosa and changes in the connective tissue, would be avoided when using a COCP with anti-androgenic and anti-mineralocorticoid properties. A less erratic voice allowed better voice control throughout the whole cycle, independently of the phase of the cycle, and this, in turn, seemed to improve confidence levels, allowing singers to explore the other two components of their performances, i.e. emotional expressiveness and musicality.

Taking into account the findings of these recent investigations, the current study aims to assess whether the use of this specific COCP containing drospirenone might also contribute to the stabilisation of vocal fold's vibratory pattern, and thus the optimisation of vocal performance of other professional female voice users, namely singers of other musical genres, choir members and teachers.

Methodology

Participants and recruitment

We studied 20 healthy female voice users (mean age = 26.60; SD = 6.319; range = 19-40 years), all living in Sheffield, England. All participants were healthy volunteers, using their voices professionally: seven of these participants were music theatre soloists; two were professional jazz singers; five were choir members; and six were school teachers.

The participants were recruited by means of: (i) a webpage designed to advertise the study (www.sheffield.ac.uk/music/research/voiceresearch); (ii) a press release done by the University Press Office advertising the study; (iii) a brief news in a local news paper advertising the research; (iv) a letter sent to several primary and secondary schools in Sheffield; (v) leaflets distributed amongst several schools in Sheffield, University Departments, and choirs; (vi) oral presentations about the study, delivered at primary and secondary schools in Sheffield. We were able to recruit a total of twenty seven participants; however, seven participants fallen to complete the study immediately before the first recording session for personal reasons.

After the participants volunteered, a meeting with the consultant in gynaecology involved in this study (W.L.) was arranged to guarantee the participant's suitability to take part in the

study. All recruited participants had the criteria to be included in this study, i.e.: (i) were healthy women; (ii) had normal blood pressure; (iii) did not have history of breast, ovarian and cervical cancer; (iv) were not pregnant, or wish to be pregnant; (v) had no gynaecological/endocrinological problems; (vi) had regular menstrual cycles; (vii) were regularly using their voices, professionally or semi-professionally; (viii) did not report a history of vocal problems.

Study protocol

In order to assess the effects of this low dose COCP (Yasmin - 30μg ethinylestradiol and 3mg drospirenone - Schering AG, West Sussex, UK) on the pattern of vibration of the vocal folds, we designed a cohort study in which each participant was studied during two consecutive months: the first month not using a COCP, and the second month using a COCP. For both arms of the study (i.e. natural and COCP cycles), vocal and blood samples were collected at three specific points of the menstrual cycle: menstruation, and during the follicular and luteal phases of the menstrual cycle.

Because every institution that conducts or supports biomedical or behavioural research involving human participants must have an initial approval and periodical reviews to protect the rights of human participants, the whole protocol and experimental design were approved by the Ethics Committee of the University of Sheffield previous to the beginning of the study.

Procedure

After ensuring that participants were suitable for the study, arrangements were made to plan data collection sessions. In the first part of the study, participants were not using a COCP: the first data collection session was carried out on the second day of bleeding, and the two

following sessions were carried out on the 11th day of the cycle (i.e. 11 days after the first day of bleeding) and on the 24th day of the cycle (i.e. 24 days after the first day of bleeding). This was possible because participants had regular menstrual cycles, of approximately 28 to 30 days length. Each data collection session consisted of audio-electrolaryngographic recordings of the voice and a blood sample.

The audio-electrolaryngraphic recordings were carried out with the new digital Laryngograph® (Laryngograph Ltd.), which consists of a Laryngograph® processor, connected to two plated electrodes, an omindirectional (pressure sensitive) electret EK series microphone, with +/- 2dB 100Hz to 10kHz noise level 26dB (SPLA), and dynamic range of 88dB, and linked via a USB interface to a portable Pentium-M (Centrino) processor, 512 MB RAM and 80GB IDE HDD.

For each recording session, participants were asked to:

a) read a standard text, the "rainbow passage";

b) to sing a song, "Yesterday", by The Beatles, meanwhile hearing the respective piano accompaniment in one head-phone;

The blood samples were collected at the end of the recording session using: medi swab IPA cleaning pad; cotton wool balls; dental rolls; sharps bin for disposal used needles; plasters and micro-pore tape; vacutainer barrels; 22G vacutainer needles; vacutainer bottles, containing an activator and separator gel; tourniquet; small pillow. These samples were collected by a fully authorised and trained phlebotomist.

In the second part of the study, the consultant in gynaecology involved in this study (W.L.) distributed one month of COCP amongst participants, giving them verbal and written

information on how to use this medication. On the first day of bleeding, after the first month of the study (i.e. the natural menstrual cycle), the participants started this medication. The same methodology as for the first part of the study was used and data were collected on the same days of the COCP cycle as for the natural menstrual cycle. Datasets were always collected in the same room of the Music Department of the University of Sheffield.

Vocal analysis

Vocal changes associated with the menstrual cycle have been explained as a consequence of abnormalities in the vocal fold vibration pattern due to sex hormonal variations. Oedema of the vocal fold mucosa, an increased number of microvaries, and aggravations of gastroesophageal reflux with consequent vocal laryngitis, are examples of some of the phenomena that have been related to premenstrual and menstrual phases of the cycle, especially in women presenting luteal insufficiency.[13] Taking into account the above symptoms, and because *"…the crucial event for voice production is vibration of the vocal folds…"* (quoted from Hirano, 1981: 3), vocal analysis in this research are mainly concerned with variations in the pattern of vibration of the vocal folds. Because these variations can be assessed by studying vocal fold [14], the following electrolaryngographic parameters were analysed using the Laryngograph PCLX software ("Speech studio' - *Spead* and *Qanalysis*): irregularity of vocal fold vibration, based on (a) period-to-period frequency peaks (CFx), and on (b) period-to-period amplitude peaks (CAx), in the cycle to cycle excitation of the vocal tract. The study of irregularity of vocal fold vibration can be used as a direct means of cognitive control of voice production, and hoarseness.[15] CFx is normally used to assess period-to-period irregularities, and it can be seen as a means of jitter measurement, applied to connected speech and sounds, instead of

the commonly used sustained sounds analysis for jitter extraction. Although the human voice shows some irregularity in the vibration of the vocal folds, pathologies such as mucosal oedema are expected to produce more irregularity, and thus higher CFx values (higher than approximately 6%).[19] CAx is normally used to assess hoarseness and huskiness, symptoms that have been associated with vocal fold oedema and enlarged microvarices with possible vocal haemorrhage.[16, 17] CAx comes from irregularity of successive amplitude peaks in the cycle to cycle excitation of the vocal tract. Voices that are hoarser are expected to have higher CAx values (higher than approximatelly 6%).[19]

Assays

Sex steroid hormones, i.e. oestrogens, progestogens and testosterone, are mainly produced in the gonads (testes for males, and ovaries for females).[22] Oestradiol is the principal and most potent oestrogen produced by the ovaries. It regulates the secretion of gonadotropins during the menstrual cycle, and causes proliferation of the endometrial, vaginal and urinary tract mucosa.[24] It is also responsible for thinning the cervical mucus and promotes the development of secondary sex female characteristics.[23] Progesterone counteracts the effects of oestradiol, i.e. it increases the rate of degradation and inactivation of oestradiol and decreases the number of oestradiol receptors. It avoids the proliferation of cells of the mucosas and decreases glandular activity. Mucus secretion is therefore decreased and the mucus becomes thick and opaque. Because progesterone decreases the blood vessels' permeability, it tends to cause fluid retention in the ground substance, thus causing tissue oedema.[24] Testosterone in women is secreted not only by the ovaries, but also by the adrenal glands and the peripheral compartment.[21] Total concentrations of testosterone, translated as a percentage, i.e free androgen index (FAI), can be calculated by measuring the amount of free and bound testosterone.

On these grounds, the following analyses were taken in order to study variations in the concentrations of the above mentioned hormones: i) the IMMULITE Analyzer for *in vitro* quantitative measurement of oestradiol, and progesterone in serum; ii) the ADVIA Centaur™ System for *in vitro* quantitative measurement of total testosterone (bound and unbound[1]) in serum. All blood samples were analysed in the Clinical Laboratory of the Department of Clinical Chemistry, at the Royal Hallamshire Hospital, and the results collected later.

Statistical analysis

Descriptive statistics including means, standard deviations and proportions were used to analyse the hormonal concentrations (i.e. oestradiol; progesterone; testosterone; and free androgen index) and vocal fold irregularity (i.e. CFx and CAx) of both natural and COCP cycles, for each phase of the menstrual cycle (i.e. menstrual, follicular and luteal). An analysis of variance was carried out - fitting effects for subject, time and pill use separately by each phase of the cycle. This model assumes that the residuals from such a model are normally distributed. We checked for normality by using histograms of the residuals. We regarded a p value of < 0.05 as statistically significant. All statistical analyses were carried out using SPSS v12.

Results

We present results of blood analyses and ELG recordings for the 20 subjects in the menstrual, follicular and luteal phases of the menstrual cycle.

Table 1 shows the means and standard deviations for hormonal concentrations, percentage of irregularity in the frequency of vibration of the vocal folds (CFx), and percentage of

[1] Some sex hormones, such as testosterone and oestrogen, can exist free in the body or bound to a protein which carries it in the blood (Immulite SHBG, PILKSH-5, 2002: 2).

irregularity in the amplitude of vibration of the vocal folds (CAx), during the menstrual phase of the menstrual cycle, for both no COCP and COCP conditions, and the results of the repeated measures one way ANOVA tests, for both reading and singing tasks.

(please insert Table 1 about here)

For this phase of the menstrual cycle, no significant differences were found for both natural and COCP cycles for CFx and CAx percentages, and for both reading and singing tasks. Significant differences were found for concentrations of FAI values [$F(1) = 18.013$; $p = 0.000$]. FAI percentages were significantly higher for the natural menstrual cycle.

Table 2 shows the means and standard deviations for hormonal concentrations, percentage of irregularity in the frequency of vibration of the vocal folds (CFx) and percentage of irregularity in the amplitude of vibration of the vocal folds (CAx), during the follicular phase of the menstrual cycle, for both no COCP and COCP conditions, and the results of the repeated measures one way ANOVA tests, for both reading and singing tasks.

(Please insert Table 2 about here)

No significant differences were found for the vocal parameters assessed between the two conditions of this study and for both reading and speaking tasks. Significant differences were found between the natural and COCP cycles for concentrations of testosterone [$F (1) = 11.721$; $p = 0.003$], and values of FAI [$F (1) = 17.029$; $p = 0.001$]. Concentrations of testosterone and FAI percentages were significantly higher during the natural cycle.

Table 3 shows the means and standard deviations for hormonal concentrations, percentage of irregularity in the frequency of vibration of the vocal folds (CFx), and percentage of irregularity in the amplitude of vibration of the vocal folds (CAx), during the luteal phase

of the menstrual cycle, for both no COCP and COCP use conditions, and the results of the repeated measures one way ANOVA tests, for both reading and singing tasks.

(Please insert Table 3 about here)

Once again no significant differences were found for the vocal parameters assessed for both reading and singing tasks. Significant differences were found for concentrations of oestradiol, progesterone, testosterone and FAI vales. Significantly higher concentrations were found during the natural menstrual cycle for oestradiol [F (1) = 90.105; p = 0.000], progesterone [F (1) = 26.707; p = 0.000], testosterone [F (1) = 6.636; p = 0.019], and values of FAI [F (1) = 15.539; p = 0.01].

Discussion

In summary, the results suggest that significant differences were found between hormonal concentrations during the natural and COCP cycles; however, no significant differences were found in vocal fold's irregularity and contact area during vocal performance between these two conditions of the study.

Hormonal analyses confirm the results of previous studies suggesting that the use of this COCP has anti-androgenic effects on the body. Significant lower values of testosterone during the follicular and luteal phases of the cycle, and lower free androgen index throughout the whole cycle suggest that this COCP presents anti-androgenic even with only one month of use. In addition, and according to the expectations, the significant lower concentrations of oestrogen and progesterone during the luteal phase of the COCP cycle showed contraceptive effectiveness. [a]

Concerning the vocal results obtained for the **reading sample**, the high mean values of CFx, AND CAx suggest that the participants had poorer speaking voices, independently on COCP use. The mean values of CFx and CAx were majority higher than 6% for the three phases of the menstrual cycle. According to Fourcin, McGlashan & Blowes (2002), although the human voice shows some irregularity in the vibration of the vocal folds, pathologies such as mucosal oedema are expected to produce more irregularity, showing CFx and CAx values above 6%.[15] It is possible that the high CFx, and CAx values obtained might be related to the fact that these women might suffer from vocal misuse and abuse related to their jobs rather than COCP use. Teaching usually is associated with many hours of speaking and sometimes even loud speaking; music theatre singers are known to place great demands on their vocal folds as they extend their "chest voice" registers to higher notes; choristers do not have a deep knowledge of vocal technique, which some times can lead to vocal misuse; jazz singers normally sing in smoky environments, and also do not have such vocal preparation as other singers. Therefore, one might expect that vocal situations as vocal fold oedema are common amongst these professional voice users who have these poor vocal backgrounds and place great demands on their vocal mechanism.

Looking at the results obtained for the **singing sample**, the lower mean values of CFx and CAx suggest that the pattern of vibration of their vocal folds was more regular when compared with the speaking task. Although 6 of the participants were teachers, thus not used to use their voices singing, it seems that overall singing voice was less erratic than the speaking voice. On could argue that singing could be beneficial to all professional voice users. Vocal technique might contribute to a better control and efficient care of the professional voice.

No significant differences were found concerning vocal irregularity between the natural and COCP cycles. This result could be associated with several situations:

1. The use of this specific COCP does not have an effect on the pattern of vibration of the vocal folds on professional voice users who are not classically trained singers. The performance of Western classical repertoire places greater demands on the vocal mechanism in comparison with the performance of a popular song; thus, even small differences in the pattern of vibration of the vocal folds would become more evident during the performance of classical repertoire;
2. Participants, although all using their voices as a tool of trend, placed different demands on their vocal mechanisms. Thus, results could be different if they were all music theatre singers, choir members, or teachers. However, the purposes of this cohort study were to gather pilot data on the pattern of vibration of several professional voice users when using and not a COCP preparation containing drospirenone.
3. The designs used in studies concerned with the effects of COCP use on the professional voice were all different. Previous studies have followed observational, comparative, cohort and double blind randomised placebo controlled designs. It would be important to choose similar designs and develop similar methodological approaches to study these matters in order to achieve more robust and definitive results.
4. Finally, it is also possible that a longer period of COCP use would show clear effects on the voice. However, hormonal results showed effects related to COCP use with only one month of COCP use, such as anti-androgenic and contraceptive properties. Therefore, effects somewhere else would be equally expected.

Although there were no significant differences between the natural menstrual cycle and the COCP cycle, comparisons between the results for both speaking and singing tasks suggest a trend for a slightly more erratic voice during the natural menstrual cycle: (i) differences between the natural and COCP cycles are more evident for the singing sample when compared with the reading sample; (ii) COCP use is associated with lower values of CFx, and CAx for the speaking and reading samples, and during the menstrual and follicular phases of the cycle. This trend is concordant with the results of the first double blind randomised placebo controlled trial assessing the effects of this COCP on the pattern of vibration of the vocal folds during the performance of western classical repertoire in which measures of CAx were significantly lower when using this specific COCP during the performance of Western classically repertoire.[9]

Conclusions

The results of this work suggest that the use of a COCP containing drospirenone does not have an effect on vocal regularity of professional voice users. The anti-androgenic and anti-mineralocorticoid properties of this specific COCP, previously shown to be beneficial to classically trained singers, seem not to have an affect on the voices of other singers (namely music theatre and jazz singers, and choir members), and school teachers. However, three important conclusions arose from this work:

1. It is important to use similar study designs and methodological approaches in future research concerned with the effects of hormone related medication on the professional voice in order to obtain robust and definitive results;
2. Studies assessing the effects of hormone related medication on the professional voice should be confined to specific groups of voice users within the population, according to their specific vocal use. It is possible that within the population of professional voice users, those who are classically trained singers are more prone

to be affected by even small variations in the pattern of vibration of the vocal folds when compared to singers of other music genres and other professional voice users.

3. Singing activities might be beneficial to all professional voice users, contributing to a more efficient vocal mechanism, and better vocal health protection.

We propose that future research in this field will use double blind randomised placebo controlled trials (RCT) to test the effects of hormone related medication on the voice. This type of design is thought to produce objective results, since the expectations of the researcher and the participant about the experimental drug do not affect the outcomes. Furthermore, the random allocation of the treatment and its placebo for each participant provides similar experimental conditions for all participants, so the outcomes will be equally influenced by external factors, despite the fact that there are individual differences.[27] Study designs comparing two different groups of women, COCP and non-COCP users, or non-randomised studies, are less advantageous and less powerful in terms of the reliability of their results when compared with crossed-over RCTs. The expectations from a comparison of different interventions (COCP and placebo use) on the same voice user are always more precise than a comparison between different voice users. Furthermore, the fact that in cross-over RCTs the same participant is his/her own control makes the results be more realistic even when the sample size is small.[28]

It would be important to study larger and less varied population of professional voice users. Further significant progresses in this filed of research could be achieved if studies on the effects of hormone related medication on the voice would be conducted with teachers, or singers. Additionally, within the group of singers, these studies should narrow the research to a specific singing technique group (e.g. classically, musical theatre, jazz, etc.). Although

it is expected that professional voice users place great demands on their voices, vocal behaviour would be different to these different voice professionals.

Finally, another conclusion that could be drawn from this study is that singing might be beneficial to professional voice users. During singing, the vocal mechanism seems to become less erratic. Thus, by applying some features of singing into the speaking mechanism, professional voice users who tend to majority use their speaking voices perhaps could avoid acquiring poor habits, and develop some vocal techniques which would contribute to a more efficient speaking vocal mechanism and a better vocal health protection. Professional voice users, especially those who are teachers, constitute a large percentage of the patients who visit ENT clinics for the treatment of vocal problems. By educating them on preventing future vocal problems possibly associated with menopausal changes, this study will also contribute to better management of the socio-economic resources of the National Health Service.

Acknowledgements

The authors would like to thank the participants, the Department of Clinical Chemistry and the Pharmacy Services Directorate at the Royal Hallamshire Hospital, Schering Health Care Ltd (UK) for the donation of Yasmin®, and the White-Rose Foundation, for supporting financially this study.

References

1. Guillebaud J. Contraception: your questions answered. 3rd Edition. London: Churchill Livingstone, an imprint of Harcourt Publishers Limited, 1999.

2. Brodnitz FS. Hormones and the human voice. Bulletin of the New York Academy of Medicine 1971; 47: 183-191.

3. Dordain M. Etude statistique de l'influence des contraceptives hormonaux sur la voix. Folia Phoniatrica 1972; 24: 86-96.

4. Helmi AM, Ghazzawi IF, Mandour MA, Shehata M.A. The effect of oestrogen on nasal respiratory mucosa: an experimental histopathological and histochemical study. Journal of Laryngology and Otology 1975; 89: 1229-1241.

5. Schiff M, Burn H. The effect of intravenous estrogens on ground substance. Archives of Otolaryngology 1961; 73: 43-51.

6. Wendler J, Siegert C, Schelhorn P, Kingler G, Gurr S, Kaufmann J, Aydinlik S, Braunschweig. The influence of Microgynon® and Diane-35®, two sub-fifty ovulation inhibitors, on voice function in women. Contraception 1995; 52: 343-348.

7. Isenberg H, Brown WS, Rothman HB. Effects of menstruation on the singing voice; Part II: Further developments in research. In Laurence V (editor) Transcriptions of the twelfth symposium for the care of the professional voice. Part I. New York: The Voice Foundation, 1983.pp. 117-123.

8. Lã F, Ledger W, Davidson, JW, Howard MD, Jones G. The effects of a third generation combined oral contraceptive pill on the classical singing voice. Journal of Voice 2006; In Press.

---

11. Essman, E. J. & Abramson, A. (1984). Estrogen binding sites on membranes from human laryngeal papilloma. *International Journal of Cancer*, 33: 33-36.

12. Chae, S. W., Choi, G., Kang, H. J., Choi, J. O. & Jin, S. M. (2001). Clinical analysis of voice change as a parameter of premenstrual syndrome. *Journal of Voice*, 15: 278-283.

13. Abitbol, J. Abitbol, P. & Abitbol, B. (1999). Sex hormones and the female voice. *Journal of Voice*, 13: 424-446.

14. Cranen, B. & Jong, F. (2000). Laryngostroboscopy. In R. D. Kent & M. J. Ball (Eds.) *Voice Quality Measurement*. (pp. 257 – 267). San Diego: Singular Thompson Learning.

15. Fourcin, A., McGlashan, J. & Blowes, R. (2002). Measuring voice in the clinic – Laryngograph Speech Studio Analysis. *Presented at the 6th Voice Symposium of Australia*. Retrieved from http://www.laryngograph.com/ papers.htm [internet access 4 November 2004].

---

18. Howard, D. M. (1995). Variation of electrolaryngographically derived closed quotient for trained and untrained adult female singers. *Journal of Voice*, 9: 163-172.

19. Laryngograph Ltd., Speech studio user's guide. Retrieved from http://www.laryngograph.com/ pr_studio.htm [internet access 29 January 2003].

20. Titze, I. R. (2000). *Principles of Voice Production*. 2nd Edition. Iowa: National Center for Voice and Speech.

21. Carmina, E. & Lobo, R. A. (2004). Evaluation of hormonal status. In J. F. Strauss & R. L. Barbieri (Eds.) *Yen and Jaffe's Reproductive Endocrinology.* 5th Edition (pp. 939-964). Philadelphia: Elsevier Saunders.

22. Rehn, T. & Cidlowski, J. A. (2004). Steroid hormone action. In J. F. Strauss & R. L. Barbieri (Eds.) *Yen and Jaffe's Reproductive Endocrinology.* 5th Edition (pp. 155-174). Philadelphia: Elsevier Saunders.

23. Bulun, S. E. & Adashi, E. Y. (2003). The physiology and pathology of the female reproductive axis. In P. R. Larsen, H. M. Kronenberg, S. Melmed & K. S. Polonsky (Eds.) *Williams Textbook of Endocrinology*. 10th Edition. (pp. 587-664). Philadelphia: Saunders.

24. Amir, O. & Biron-Shental, R. (2004). The impact of hormonal fluctuations on female vocal folds. *Current Opinion in Otolaryngology and Head and Neck Surgery*, 3: 180-184.

25. Stewart, P. M. (2003). The adrenal cortex. In P. R. Larsen, H. M. Kronenberg, S. Melmed & K. S. Polonsky (Eds.) *Williams Textbook of Endocrinology*. 10th Edition. (pp. 491-551). Philadelphia: Saunders.

26. Strauss, J. F. (2004). The synthesis and metabolism of steroid hormones. In J. F. Strauss & R. L. Barbieri (Eds.) *Yen and Jaffe's Reproductive Endocrinology.* 5th Edition (pp. 125-154). Philadelphia: Elsevier Saunders.

(Conclusion section)

27. Pocock, S. T. (1983). *Clinical trials: a practical approach*. Singapore: John Wiley & Sons.

---

28. Lowe, D. (1993). *Planning for medical research: a practical guide to research methods*. Middlesbrough: Astraglobe Limited.