Title: Fundamental frequency variations across the menstrual cycle and the use of an oral contraceptive pill use

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ABSTRACT

Purpose: Concentrations of sex steroid hormones – estrogens, progesterone and testosterone - have been associated with premenstrual and menstrual vocal symptoms. However, the extent to which these symptoms may be reflected on acoustical features of the voice is still debated. This study investigates variations in fundamental frequency (f_o) and related parameters in connected speech across phases of the menstrual cycle and during the use of a combined oral contraceptive pill (OCP).

Method: Electrolaryngographic recordings were made and blood samples collected at three different phases of the menstrual cycle – menstrual, follicular and luteal - for placebo and OCP use. These two conditions were blindly and randomly allocated in the study. Speaking fundamental frequency (SFF), SFF standard deviation, SFF rate of change, SFF slope, maximum and minimum f_o, and f_o range were extracted for nine healthy females while reading a phrase from the Rainbow Passage. Concentrations of sex hormones were analyzed in serum. Non-parametric statistical tests were carried out to assess differences between phases and conditions.

Results: SFF, its standard deviation and maximum f_o were significantly different between phases of the menstrual cycle for placebo use only. Menstrual phase showed the lowest values. Maximum and minimum f_o were significantly different between placebo and OCP use for menstrual and follicular phases, respectively.

Conclusions: Fluctuations in sex steroid hormones across the menstrual cycle alter f_o in speech more than a particular hormonal concentration. OCP use seems to have a stabilizing effect on the voice relative to f_o and related parameters in speech.

INTRODUCTION

Sex steroid hormones affect the voice [Abitbol, Brux, Millot, Masson, Mimoun, Pau & Abitbol, 1989]. Through life, a person's voice undergoes changes that seem to follow the variations in concentrations of sex steroid hormones (i.e., estrogens, among which estradiol – E2 - is the most influential, progesterone – P - and testosterone - T). In males, the voice changes in early and later stages of biological development (i.e., puberty and andropause). In females, due to the complexity of their reproductive endocrine system (leading to monthly variations of sex hormones), the voice is notably more effected across the whole reproductive lifespan [Lã & Sundberg, 2012].

About one third of women have complained of pre-menstrual and menstrual vocal symptoms, including vocal fatigue, decreased range and hoarseness [Abitbol et al., 1989; Amir & Biron-Shental, 2004]. Such symptoms can be explained by the findings of earlier investigations: the histological dependences between sex steroid hormones and the tissues of both cervical mucosa (in the neck of the womb) and vocal folds mucosa are similar [Perelló & Comas , 1959; Abitbol et al., 1989; Abitbol, Abitbol & Abitbol, 1999]. In addition, receptors for sex steroid hormones have been found in several subunits of the vocal folds [Essman & Abramson, 1984; Newman, Butler, Hammond & Gray, 2000; Schneider, Cohen, Stani, Kolbus, Rudas, Horvat & van Trotsenbur, 2007; Voelter, Kleinsasser, Joa, Nowack, Martínez, Hagen & Voelker, 2008], the highest numbers found in the vocal ligament and *maculae flavae* [Kirgezen, Sunter, Yigit & Huq, 2017]. Thus, one expects that bodily changes associated with the menstrual cycle can also be expressed as changes in physical properties of the vocal folds. The estrogenic effects on epithelium thickening and on increased vascularity can both account for vocal fold edema and development of mucosal microvarices.
Conversely, the progestogenic effects on mucous production and its thickening would explain the increased frequency of throat clearing [Abitbol et al., 1999; Abitbol, 2006].

Although the impacts of sex steroid hormones across the menstrual cycle on perceived vocal symptoms and on physical properties of the vocal folds have been acknowledged in several previous studies, the extent to which these symptoms are manifested on the acoustic properties of the voice is still debated, specially concerning fundamental frequency (f_o) in speech.

It has been hypothesized that vocal fold mass increases due to edema and vascular changes associated with pre-menstruation (luteal phase) and menstruation (menstrual phase). These conditions would be responsible for a decrease in mean f_o during these phases of the menstrual cycle [Frable, 1962]. However, if such effect on f_o was observed in previous investigations [e.g., Molina, Brasolotto, Berretin-Felix & Cristovam, 2000; Tatar, Sahin, Demiral, Bayir, Saylam & Ozdek, 2016], others have failed to replicate it [e.g., Silverman & Zimmer, 1978; Wilson & Purvis, 1980; Chae, Choi, Kang, Choi & Jin, 2001; Çelik, Çelik, Ateşpare, Boyacı, Çelebi, Gündüz, Aksungar & Yelken, 2013; Kunduk, Vansant, Ikuma & McWhorter, 2017]. To add to this controversy, an increase in mean f_o was found when concentrations of estradiol were higher (i.e., near ovulation during later follicular phase) [Raj, Gupta, Chowdhury & Chadha, 2010; Fischer, Semple, Fickenscher, Jürgens, Kruse, Heistermann & Amir, 2011; Arruda, Diniz da Rosa, Almeida, de Araujo Pernambuco & Almeida, 2019].

Different methodological approaches and limitations of study designs may have contributed to the lack of agreement in these previous investigations. On the one hand, most studies have extracted mean f_o from audio signals of sustained vowels, whereas few have extracted it from connected speech [Gorham-Rowan, Langford, Corrigan & Snyder, 2004; Meurer, Fontoura, Corleta & Capp, 2015]. The extraction of acoustical parameters from sustained vowels and connected speech provide different results: f_o values seem to be task dependent [Guimarães & Abberton, 2005]. Sustained vowels seem to be more hormonal dependent than speech

[Meurer et al., 2015]; however, connected speech provides more complete acoustic information than sustained vowels [Moon, Chung, Park & Kim, 2012]. On the other hand, audio signals are not completely free from acoustical artifacts related to microphone placement and room acoustics [Baken & Orlikoff, 2000]. To add to these drawbacks, different data collection points were used across the menstrual cycle, all lacking hormonal status confirmation in serum.

The current study investigated f_o and related parameters in connected speech across the menstrual cycle and when using a combined oral contraceptive pill (OCP). As all previous studies have used a between-subjects study design and f_o measures extracted from audio recordings, we designed a within-subjects double-blind randomized placebo-controlled trial, extracting f_o measures from electrolaryngographic (ELG) recorded signals of female voices during connected speech. The rationale for such design is three-fold. First, cross over between-subjects with random placebo/OCP allocation is a robust study design that minimizes the possibility of type II errors [Lã, Ledger, Davidson, Howard & Jones, 2007]. Second, due to the evidence that f_o variations across the menstrual cycle are caused by changes in physical properties to the vocal folds, we extracted f_o from ELG signals. ELG signals are impervious to background noise and capture vocal fold vibratory timing via electrodes at the surface of the neck. Last, there is evidence that voice changes across the menstrual cycle are caused by changes in concentrations of estrogens and progesterone. Therefore, OCP was used as a means to control hormonal variations and directly compared to placebo use. We hypothesized that OCP use will reduce hormonal variations across the menstrual cycle when compared to hormonal fluctuations during the menstrual cycle. Further, we expect that f_o variations observed during the menstrual cycle will not be present when using OCP.

METHODS

Participants and study design

The original data were drawn from a prospective study investigating the effects of OCP use on the vibratory pattern of the vocal folds during singing [Lã et al., 2007]. Participants, nine white European women (mean age = 23.1 yrs.; SD = 2.183; age range = 21 - 27 yrs.), all classically trained singers, were recruited from different higher education institutions in the UK. Participants had a previous consultation with a Gynecologist to ensure that they met the inclusive criteria (i.e., had normal and regular menstrual cycles), were suitable for OCP use, and were healthy, non-smokers and with no history of vocal pathology. Ethical approval was obtained from South Sheffield Ethics Committee prior to the beginning of the study.

For all participants, both voice recordings and blood samples were taken at the third month of placebo and the third month of OCP use, for three specific phases of the menstrual cycle: menstrual (M), follicular (F) and luteal (L) phases. Data were collected at the third month of placebo/ OCP use due to the fact that OCP effects reach a steady state after a minimum of third months intake [Lã et al., 2007]. As five singers were using an OCP prior to the beginning of the study, they were asked to interrupt the use of this medication for one month prior to the beginning of the study. Such procedure would ensure a wash-out period for oral hormonal medication prior to the beginning of the study. The study conditions (i.e., placebo and OCP), were double-blind allocated so that half of the group was randomized to start with three months of placebo, and the other half with three months of OCP. Both placebo and OCP conditions were taken consecutively for a total of 6 months. OCP was taken for 21 consecutive days with a 7-day interval between packs. The same applied to the placebo intake. To ensure correct daily use of all pills, provided in six separated packs (one per month), participants were asked to fill in a daily calendar, marking days of pill intake. This calendar was returned at the end of the study. The study started on the first day of the menstrual cycle, when the first pill of the six identical packs was taken. None of the participants became pregnant during the study.

The choice of OCP preparation (i.e., Yasmin, by Bayer Schering Pharma) was based on the fact that it contained low doses of synthetic hormones (30μg of ethinylestradiol and 3 mg of drospirenone). These characteristics have been related to reports of good toleration, with fewer risks of side effects due to antiandrogenic and antimineralocorticoid properties of drospirenone [Huber, Foidart, Wuttke, Merki-Feld, The, Gerlinger, Schellschmidt & Heithecker, 2000].

Recordings and procedures

Participants were asked to read a standard phonetically balanced text commonly used to study acoustic properties in speech (e.g., f_o), the Rainbow Passage [Fairbanks, 1960]. The choice of task was related to the fact that more acoustic information is provided by connected speech as compared to sustained vowels [Moon et al., 2012].

Simultaneous recordings of audio and ELG signals were made. A MBNM550E-L omnidirectional microphone (Canford Audio, Washington, Tyne and Wear, UK) was placed off-axis 30 cm from the lips, connected to an Alice mic-amp-pak1 microphone preamplifier (Alice Soundtech Ltd., Surrey, UK) to capture the audio signal. A laryngograph microprocessor (Laryngograph Ltd., London, UK) connected to an oscilloscope was used to collect ELG signals. The laryngograph microprocessor comes with two-neck electrodes that must be held in place externally around the larynx notch by an elastic neck band to capture the ELG signal. Appropriate electrode's placement was monitored by a visual display in the oscilloscope. Both audio and ELG signals were recorded using a TCD-D7 two channel stereo digital audio tape recorder (DAT) (Sony, Tokyo, Japan). Recordings were made at a sampling rate of 22050 Hz.

At the end of each recording session, blood samples were collected to ensure correct use of both OCP and placebo, and that data were collected during M, F and L phases of the cycle. To measure concentrations of E2 and P in serum, the IMMULITE analyzer for in vitro was used. To analyze concentrations of T in serum, the ADVIA Centaur System for in vitro was used. Further descriptions on the procedures used to analyze hormonal concentrations can be found elsewhere [Lã et al., 2007].

Data were collected at M, F and L phases for both placebo and OCP conditions. Recording days where scheduled according to the participants' reports on first day of the menstrual cycle. Recordings were done at day one or two of the cycle - representative of the M phase, and at days 8, 9 or 10 and 22, 23, 24, 25, 26 or 27 (depending on individual cycle length) - representative of the F and L phases, respectively. Such data collection schedule was possible as all participants had regular menstrual cycles. In addition, there were two menstrual cycles prior to the one for which data were collected, allowing a confirmation of cycle length for each participant. Moreover, phases of the menstrual cycle (M, F, L) were confirmed *a posteriori* with the results of the blood samples in serum. If hormonal status did not corroborate phase of the menstrual cycle and study condition, data were dismissed from analysis.

Voice analysis

The phrase - "*People look, but no one ever finds it*" - was selected from the Rainbow Passage [Faibanks, 1960] because besides allowing f_o extraction in speech, it is located within approximately 1/3 of the whole text. This would ensure that the reader would have sufficient time to get accustomed to the task without lacking attention nor being tired. For the purpose of f_o extraction, only the last portion of the intonational phrase was analyzed – "…*but no one ever finds it*". It is the later portion of an utterance that yields the highest pragmatic and linguistic information while still reflecting the participant's phonoarticulatory behavior [Beckman & Pierrehumbert, 1986]. Connected speech was analyzed instead of sustained vowels as f_o extracted this way is more representative of an individual's habitual pitch as in sustained phonation [Baken & Orlikoff, 2000]. The analysis of f_o parameters was made using ELG signals as these provide measurements voided of effects of environmental noise and room acoustics on f_o extracted measures [Baken & Orlikoff, 2000]. Extraction of f_o was completed by means of the *correlogram* module available in the custom-made software *Sopran* (by SG). This tool displays a three-dimensional graph showing the periodicity characteristics of the voice. The f_o is traced manually and the software extracts f_o values within the traces corresponding to the highest correlation, thus being free from an automatic selection mechanism of f_o value and perturbation measures [Granqvist & Hammaberg, 2003]. The output is a .smp file with an f_o curve from which f_o values can be exported to an excel table (Figure 1).

< Please insert Figure 1 about here >

Speaking f_o and related parameters

To examine possible effects of sex steroid hormones during the menstrual cycle and the use of an OCP on f_o in speech, f_o parameters related to speech production were measured. These included speaking f_o (SFF), its variation measured as the standard deviation (SFF_{SD}) and the rate of its change (SFF_{RC}) [Nilsonne, Sundberg, Ternström & Askenfetl, 1988; Lieberman, Katz, Jongman, Zimmerman & Miller, 1985]. The latter was calculated applying Nilsonne and associates' equation [Nilsonne et al., 1988], where SFF_{RC} equals the ratio between SFF_{SD} and the mean SFF (*SFF*) divided by the time window of the utterance in milliseconds (t), times 100 [Eq1].

$$SFF_{RC} = \frac{\frac{SFF_{SD}}{SFF}}{t} \times 100 \text{ [Eq1]}$$

Maximum and minimum f_o (hence Maxf_o and Minf_o), f_o range and SFF contour were also measured, the latter being extracted from SFF slope (hence SFF_{slope}) as described elsewhere [Lieberman et al., 1985].

Statistical Analysis

Due to small sample size and the within-subjects study design, nonparametric statistical analyses were made. To examine whether there was a significant difference between the three phases of the menstrual cycle (M, F and L) for each condition (placebo or OCP), a Friedman test was used for a significance level of $\alpha = 0.05$. To examine whether there was a significant difference between conditions for each phase of the menstrual cycle, a Wilcoxon signed-ranks test was used. Because this test involves three simultaneous comparisons, a Bonferroni correction was considered, and so these results were identified as significant when $p < 0.05/3 = 0.017$. All statistical analyses were made using SPSS 24.0 for Windows.

RESULTS

f_o parameters

Individual variations of SFF and related parameters can be observed in radar plots presented in Appendix 1. Table 1 summarizes descriptive statistics for each of the f_o parameters, for the three phases of the menstrual cycle and the two studied conditions, averaged across all participants. As data were not normally distributed, both median (Mdn) and interquartile range (IRQ) are reported, the latter being equal to the difference between the third and the first quartiles.

< Insert Table 1 about here >

Testing the first null hypothesis - phases of the menstrual cycle are equal for both placebo and OCP conditions, $H0_1$: M = F = L, placebo | $H0_1$: M = F = L, OCP - the results of the Friedman test show that SFF, SFF_{SD} and Maxf_o are significantly different between the three phases of the menstrual cycle for the placebo condition only. Considering only the condition for which significant differences were found (i.e., placebo use), M reveals the lowest values for all parameters. SFF was 3.4 and 13.4 Hz lower in M as compared with F and L phases, respectively [$\chi^2(2, N=9) = 10.667$, p = 0.005]. As differences in Hz represent a linear progression and the ear responds nonlinearly to pitch intervals [Hudson & Holbrook, 1981], these differences are also be expressed in semitones (ST), calculated using the following equation [Eq2], where Ref f_o = 220 Hz [Goy, Fernandes, Pichora-Fuller & Lieshout, 2013].

$$1Hz = 12 \times \frac{\log(\frac{fo}{Ref\ fo})}{\log(2)} \text{ [Eq. 2]}$$

Applying this formula, SFF was 0.3 and 1.5 ST lower in M as compared with F and L phases, respectively, for placebo use. No significant differences were found for OCP use [$\chi^2(2, N=9) = 0.222$, p = 0.895]. To what concerns SFF_{SD}, M shows values between 11.89 and 9.51 Hz (7.5 and 6.2 ST) lower than F and L phases, respectively, for the placebo condition [$\chi^2(2, N=9) = 6.899$, p = 0.032]. No significant differences between phases were found for OCP use [$\chi^2(2, N=9) = 0.889$, p = 0.641]. With respect to Maxf_o, M phase was 19 and 22.3 Hz (1.3 and 1.5 ST) lower than F and L phases, respectively, also for placebo use [$\chi^2(2, N=9) = 8.000$, p = 0.018]. Once again, no significant differences were found for OCP use [$\chi^2(2, N=9) = 0.222$, p = 0.895].

Testing the second null hypothesis - conditions are equal for each phase of the menstrual cycle, $H0_2$: Placebo = OCP, M | $H0_2$: Placebo = OCP, F | $H0_2$: Placebo = OCP, L - significant differences were found for Maxf_o only during the M phase, with OCP use showing the highest values [M: z = - 2.666; p = 0.008; F: z = - 0.059, p = 0.953; L: z = - 0.889, p = 0.374].

Significant differences were also found for Minf_o only during F phase, once again with OCP use revealing the highest values [M: z = - 0.178, p = 0.859; F: z = - 2.547, p = 0.011; L: z = - 0.533, p = 0.594]. Maxf_o was 22.2 Hz (1.5 ST) higher in OCP as compared to placebo for the M phase, whereas Minf_o was 23.02 Hz (2.6 ST) higher in OCP as compared to placebo for the F phase.

As with regard to the other SFF related parameters (i.e., SFF_{RC}, f_o range, and SFF_{Slope}), no significant differences were found neither between phases nor between conditions. A summary of the statistical results obtained can be found in Table 2.

< *Insert Table 2 about here* >

Sex steroid hormones

Concerning concentrations of sex steroid hormones, descriptive statistics were previously reported elsewhere, in terms of means and standard deviations [Lã et al., 2007] and in terms of median and interquartile range [Lã, Sundberg, Howard, Sa-Couto, & Freitas, 2012]. Figure 2 represents variations in concentrations of E2 (red), P (blues) and T (green) collected in this study, represented as bullet points for the placebo condition (left panel) and for the OCP condition (right panel). These values are plotted against normative data taken from natural regular menstrual cycles (represented with solid lines). E2 and P normative data were extracted from Stricker and associates [Stricker, Eberhart, Chevailler, Quinn., Bischof & Stricker, 2006]; and T data were extracted from normative values provided by Bui and associates [Bui, Sluss, Blincko, Knol, Blankenstein & Heijboer, 2013]. As observed in Figure 2, our data is representative of the three phases of the cycle and of the two conditions. As expected, sex steroid hormonal fluctuations across the menstrual cycle were dampened during OCP use (see Figure 2).

< *Insert Figure 2 about here* >

DISCUSSION

The primary purpose of this study was to investigate variations of f_o in connected speech (hence SFF and related parameters) in relation to sex steroid hormones during the menstrual cycle and OCP use. To achieve this aim, a double-blind randomized placebo-controlled trial was carried out with nine females. To provide robust analysis of f_o, ELG signals were analyzed in a intonational phrased extracted from a reading passage, at three phases of the menstrual cycle (M, F and L) for placebo and OCP conditions. Phases of the cycle and correct use of placebo and OCP were confirmed with hormonal concentrations in serum. The values obtained follow normative data for the three phases of regular menstrual cycles, and hormonal fluctuations were dampened during OCP use.

One would expect changes in physical properties of the vocal folds to be associated with E2 concentrations and its hypertrophic effects on mucosal cells, or to be associated with P concentrations and its related mucosal secretion thickening [Amir & Biron-Shental, 2004]. In the current study, concentrations of E2 and P were high during the L phase (also called premenstrual phase). However, significant differences in SFF, SFF_{SD} and Maxf_o between phases pointing out M phase as the responsible for such differences. For this phase, both E2 and P were significantly reduced [Lã et al., 2012]. Moreover, when comparing placebo and OCP for each phase of the cycle, significant differences in Maxf_o and Minf_o were found only for M and F phases, respectively. For these phases, f_o extreme values fluctuated lesser when sex steroid hormonal variations were damped with OCP use. Such result substantiates previous claims that voice changes across the menstrual cycle are related to constant fluctuations in sex steroid hormones rather than to concentrations of a given sex steroid hormone in a particular moment of the cycle [Abramson et al., 1984; Lã et al., 2007].

During OCP use, concentrations of all hormones were considerably reduced [Lã et al., 2012]. Comparisons between the three phases of the menstrual cycle when using an OCP revealed no differences in sex steroid hormones between phases, except for P, which was slightly higher during the F phase as compared to the other phases of the menstrual cycle [Lã et al., 2012]. These results confirm the contraceptive effects of OCP and also its stabilization effect on sex hormonal fluctuations across the menstrual cycle [Speroff, Glass & Kase, 1989]. This stabilizing effect seems to be reflected also on SFF and related parameters - SFF_{SD}, SFF_{RC}, Maxf_o, Minf_o, f_o range and $SFFS_{lope}$ – for which differences between phases could not be found. Based on the reports of previous investigations, changes during premenstrual and menstrual phases of the cycle due to effects of E2 and P on mucosal thickness, vascularity and quality of mucous production would be expected [Abitbol, et al., 1999]. If the hormonal shifts that characterize the menstrual cycle are assumed to be responsible for such vocal changes, one would expect that the dampening of these hormonal variations during OCP use would circumvent changes in voice production, and thus on acoustical characteristics of the voice, such as f_o and related parameters. These expectations are confirmed by the results here presented. They are further substantiated also by the results of the prospective study from which these data were originally collected. A more regular pattern of vibration of the vocal folds was also found in terms of amplitude of vibrations during singing for OCP use [Lã et al., 2007]. Furthermore, comparisons of f_o in connected speech between OCP and non-OCP users revealed no differences between phases of the menstrual cycle for the OCP group only [Meurer et al., 2015; Rodney & Sataloff, 2016].

At the end of the study, participants were asked to guess OCP and placebo randomization. Five singers were able to guess correctly their randomization for OCP use. This rate of correct guessing (55%) falls within the percentage reported in previous double-blind randomized placebo-controlled trials considered as valid with respect to integrity of participants' blindness (e.g., [Fairbairn, Dundon, Xie, Plebani, Kampman & Lynch, 2008].

The participants in the current study were classically trained singers. Due to the great demands that these professionals place on their voices, the study of such sample could question whether similar results would be obtained if other professional voice users' groups or the general population were investigated. However, SFF values fall within the range of values for an age-matched group of women (20 to 32 yrs.) who were not singers nor professional voice users [Ma & Love, 2010].

One may also argue that the results of the current investigation could be related to a small sample size and to a great individual variability. Nevertheless, possible impacts of these pitfalls on the results have been surpassed by the robustness of data collected in a double-blind randomized placebo-controlled trial.

A substantial variability in SFF_{SD}, SFF_{RC}, SFF_{Slope} and f_o range was found between subjects. However, such variability was expected. These parameters depend on individual speech and intonational habits relative to language and so vary within a large scale of possible values for normative data. SFF and f_o extreme values are expected to vary in a much smaller window of possible normative values for female non-pathological voices [Sanchez, Oates, Dacakis & Holmberg, 2014]. SFF and f_o extreme values here discussed fall within the range of values in normative data for females with no history of voice disorders, no smoking habits and no hearing impairments [Ma & Love, 2010; Goy et al., 2013].

The results of the current study set the ground for further research concerning effects of sex steroid hormones in the voice. It seems that constant fluctuations of sex steroid hormones across the menstrual cycle are responsible for changes in acoustic parameters [Lã et al., 2007; Abramson, Steinberg, Gould, Bianco, Kennedy & Stock, 1984]. During placebo use, significant differences between phases of the menstrual cycle where found concerning SFF,

SFF_{SD} and Maxf_o. These parameters were significantly lower for the M phase as compared to the other two. However, further questions emerge from these results: i) why effects were found only for SFF, SFF_{SD} and Maxf_o and not for all f_o related parameters analyzed, including Minf_o, f_o range, SFF_{RC} and SFF_{slope}?; ii) why the menstrual phase revealed the lowest values? iii) are the effects on SFF, SFF_{SD} and Maxf_o above the threshold of becoming audible? iv) what possible factors could account for such differences?

Changes in f_o and related parameters may depend on physical properties of the vocal folds. However, changes in f_o may also occur due to changes in auditory feedback and neural motor control mechanisms [Larson, Carrell, Senner, Burnett & Nichols, 1995; Mürbe, Pabst, Hofmann & Sundberg, 2004]. In addition, auditory and neural control of the voice seem to be affected by sex steroid hormones due to interferences with laryngeal afferent and efferent neuromotor control [Isenberg, Brown & Rothman, 1983; Abramson et al., 1984; Higgins & Saxman, 1989; Whiteside, Hanson & Cowell, 2004] and by auditory functioning [Katzenellenbogen, 2000; Charitidi, Meltser, Tahera & Canlon, 2009; Al-Mana, Ceranic, Djahanbakhc & Luxon, 2010]. Moreover, sex steroid hormonal variations seem to have an impact also on cognitive function [Hampson, 1990; Solís-Ortíz, Campos, Félix & Obregón, 2009], neural excitability [Smith, Adams, Schimdt, Rubinow & Wassermann, 2002] and sensorial processes [Grillo, La Mantia, Triolo, Scollo, La Boria, Intelisano & Caruso, 2001; Eisner, Burke & Toomey, 2004; Giuffrè, Di Rosa & Fiorino, 2007]. It is beyond the scope of this investigation to determine which of the above parameters could account more for the results here obtained. However, one could speculate that effects of sex steroid hormones on auditory and neural processes responsible for f_o control systems seem to be rather prominent.

Changes in SFF and Maxf_o between phases of the cycle fell within the magnitude of 0.3 and 1.5 ST. Such values are far away from being perceptible in connected speech by a population of non-expert listeners (e.g., musicians). Audible changes of f_o have been reported to be noticeable only when bigger than 2 ST [Grawunder & Bose, 2008]. On the contrary, changes in SFF_{SD} were within the magnitude of 6.2 to 7.5 ST. These are well above the threshold of being perceptible; thus, one may argue that sex hormones have an higher impact on SFF_{SD} as compared to SFF and Maxf_o. Typically, speakers vary f_o as a function of sentence meaning (reflected in stress patterns), sentence type (e.g., declarative vs. interrogative), and affect (e.g., mood) [Gelfer & Denor, 2014].

Here, SFF_{SD} was analyzed between phases of the cycle and between conditions always for the same last portion of the intonational phrase for all speakers. As the study design involved within-subject analyzes, the factor that seems to be left alone as a possible explanation for variations in SFF_{SD} is affect. Changes in affect associated with the menstrual cycle, such as mood swings, have been described as earlier as 1937 [McCance, Luff & Widdowson, 1937]. In addition, changes in SFF and its variation have been found in depressed patients, to whom mood has been reported to be low [Nilsonne et al., 1988]. Therefore, one may speculate that sex steroid hormonal fluctuations across the menstrual cycle may be reflected to a larger extent on the way an individual uses f_o to communicate. However, such assumption is not substantiated by the results of this study. The lack of differences in SFF_{RC}, SFF_{Slope} and f_o range between phases and conditions suggest that complex interactions between kinesthetic, auditory and neural processes involved in speech production and in its control may conceal effects of sex steroid hormonal fluctuations on f_o variations during speech [Lã et al., 2012].

CONCLUSIONS

The results of this investigation suggest that constant fluctuations of sex steroid hormones across the menstrual cycle impact on SFF, SFF_{SD} and f_o extreme values, with menstruation revealing the lowest values. When hormonal fluctuations are dampened by the use of a third generation OCP, fluctuations in f_o extreme values become smaller and no differences are found for any of the f_o measured parameters in speech. Such result seems to corroborate the stabilizing effects of OCP on voice production found earlier. Because f_o production depends on prephonatory, kinesthetic and auditory/neural control, complex interactions between these factors, together with individual differences in habitual speech and phonation, may restrict the understanding of how sex hormonal fluctuations impact on physical properties of the vocal folds and related acoustic outputs. Investigating f_o variations in connected speech using ELG analysis seem to be a promising way of looking at these interactions.

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Table 1. Summary results of the descriptive statistics carried out for the speaking f_o parameters during the three phases of the menstrual cycle – menstrual, follicular and luteal – and for the two conditions (placebo and OC) for 9 participants.

Speaking *fo* parameters	**Menstrual phase**				**Follicular phase**				**Luteal phase**			
	Placebo		**OCP**		**Placebo**		**OCP**		**Placebo**		**OCP**	
	Mdn	**IQR**	**Mdn**	**IQR**	**Mdn**	**IQR**	**Mdn**	**IQR**	**Mdn**	**IQR**	**Mdn**	**IQR**
SFF [Hz]	207.7	32.7	219.8	44.1	209.4	35.6	221.1	52.9	227.8	35.0	223.0	52.3
SFF_{SD} [Hz]	20.5	10.3	27.5	9.8	34.5	16.7	26.7	15.4	28.3	12.9	21.6	7.2
SFF_{RC} [Hz/s]	73.4	42.2	112.9	73.0	129.3	83.0	99.4	39.0	103.0	49.0	100.3	28.0
Maxf_o [Hz]	246.5	30.9	267.6	65.7	274.3	79.7	261.5	49.1	269.8	43.5	257.1	52.9
Minf_o [Hz]	149.9	33.5	159.1	36.4	147.5	20.3	162.1	24.7	158.5	34.3	170.0	39.2
f_o range [Hz]	73.4	42.2	112.9	73.0	129.3	83.0	99.4	39.0	103.0	49.0	100.3	28.0
SFF_{slope}	-52.1	-19.1	-56.9	-31.3	-72.2	-34.7	-62.6	-28.9	-55.6	-68.6	-40.2	-38.7

Note. OCP = oral contraceptive pill; IQR = interquartile range; SFF = speaking fundamental frequency; SFF_{SD} = speaking fundamental frequency standard deviation; SFF_{RC} = rate of speaking fundamental frequency change; Maxf_o = maximum fundamental frequency; Minf_o = minimum fundamental frequency; f_o range = fundamental frequency range; SFF_{slope} = speaking fundamental frequency contour slope.

Table 2. Summary results of the statistical tests carried out for comparing speaking f_o related parameters between phases of the cycle and between conditions.

Hypotheses		***fo parameters***							**Test**
		SFF	SFF_{SD}	SFF_{RC}	Maxf_o	Minf_o	f_o range	SFF_{slope}	
H0: M = F = L	Placebo	0.005*	0.032*	0.169	0.018*	0.641	0.121	0.459	Friedman p < 0.05*
H0: M = F = L	OCP	0.895	0.641	0.895	0.895	0.895	0.895	0.459	Friedman p < 0.05*
H0: Placebo = OCP	M	0.173	0.021	0.051	0.008*	0.859	0.021	0.11	Wilcoxon p < 0.017*
	F	0.021	0.028	0.678	0.953	0.011*	0.139	0.086	Wilcoxon p < 0.017*
	L	0.515	0.11	0.767	0.374	0.594	0.515	0.515	Wilcoxon p < 0.017*

Note. A Friedman test was carried out to evaluate whether there are statistically significant differences ($p < 0.05$) between the three phases of the menstrual cycle within each condition (i.e., placebo or OCP), whereas a Wilcoxon Signed-ranks test was carried out to investigate whether there are statistically significant differences ($p < 0.017$) between conditions (i.e., placebo and OCP) for each phase of the menstrual cycle. H0 = null hypothesis; M = menstrual phase; F = follicular phase; L = luteal phase.

Figure 1. Output display of the custom made software *Sopran* (by SG) displaying the electrolaryngograph (ELG) signal for the intonational phrase "… but no one ever finds it" (upper panel), with the corresponding correlogram (middle panel) output and its fences placed manually delimiting the fundamental frequency (f_o) contour, which values were then extracted corresponding to the highest correlations (lower panel) for extraction of f_o in Hertz (Hz).

Figure 2. Graphical representation of sex steroid hormonal variations across the menstrual cycle. Solid lines represent normative data for a natural regular 30-day menstrual for concentrations of estradiol (red) and progesterone (blue) [Stricker et al., 2006], and for concentrations of testosterone (green) [Bui et al., 2013]. Normative data is plotted against sex steroid hormonal concentrations collected during the current investigation for placebo (left) and OCP (right) use. Estradiol, progesterone and testosterone concentrations collected during this study are represented by red, blue and green bullet points, respectively.

Appendix 1. Participants' SFF and related measures – SFF_{SD}, SFF_{RC}, SFF_{Range} and SFF_{Slope} (above), and f_o range, Maxf_o and Minf_o (below) - for each phase of the menstrual cycle (menstrual, blue; follicular, green; and luteal, blue) and the two conditions, placebo and OCP. Numbers refer to participants. Fundamental frequency values are expressed in Hertz (Hz).