Effects of the Menstrual Cycle and Oral Contraception on Singer's Pitch Control

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ABSTRACT

Purpose: Difficulties with intonation and vibrato control during the menstrual cycle have been reported by singers; however, this phenomenon has not yet been systematically investigated.

Method: A double blind randomised placebo controlled trial assessing effects of the menstrual cycle and use of a combined oral contraceptive pill (OCP) on pitch control in singing is presented. Audio-electrolaryngograph recordings were made and blood samples were taken from 9 singers in each of the three phases of the menstrual cycle both under the placebo and the OCP conditions for a total of 6 months. Participants sang an exercise consisting of an ascending octave followed by a descending major triad, starting on pitches F4 and B4. Pitch control was assessed in terms of the octave's deviations from pure intonation and of the vibrato rate and extent.

Results: Significant differences were found between the three phases of the cycle regarding octave size only for pitch F5 during OCP use. Significant vibrato rate differences between placebo and OCP conditions were found only for pitch F5.

Conclusion: OCP use may have an effect on pitch control in singers. Possible explanations point at a complex interaction between hormonal milieu and pitch control, enhancing the need for longitudinal studies.

KEY WORDS: Intonation, vibrato rate, vibrato extent, menstrual cycle, oral contraceptive pill

INTRODUCTION

Sex steroid hormonal variations are known to affect voice production. The *castrato* voice (Jenkins, 1998); puberty (Cooksey & Welch, 1998); and the menstrual cycle (Abitbol et al. 1989; Abitbol, Abitbol, and Abitbol, 1999; Lã & Davidson, 2005) constitute well known examples. However, as yet the extent to which sex steroid hormones affect singers' performances is poorly documented (Sataloff, Emerich, & Hoover, 1997; Abitbol & Abitbol, 2000).

Variations in concentrations of sex steroid hormones divide the menstrual cycle in two main phases. The first is the *follicular phase*, characterised by low concentrations of progesterone and increasing concentrations of oestrogens towards the latter part. The second is the *luteal phase*, first characterised by high concentrations in both oestrogens and progesterone, and then, in the absence of conception, by a sharp decrease in these hormones, leading to the beginning of a new cycle (Carmina & Lobo, 2004). It is these hormonal shifts that are assumed to underlie the changes in voice production. When using an oral contraceptive pill (OCP) these hormonal variations are dampened across the entire cycle (Senanayake & Potts, 1995).

Anecdotal reports by singers concerning self-perceptions of effects of the menstrual cycle on their performances highlight that, at certain phases of the menstrual cycle, specific vocal symptoms occur, e.g. hoarseness, vocal fatigue, decreased range, problems singing pianissimo, and difficulties with vibrato control and intonation (i.e. reproduction of intended pitches) (Lã & Davidson, 2005). Previous studies have found possible explanations for these vocal symptoms, but were mostly focussed on other aspects of voice production rather than intonation (Frable, 1961; Perelló & Comas, 1959; Isenberg, Brown & Rothman, 1983; Abramson *et al.*, 1984; Higgins & Saxman, 1989; Abitbol et al.

1999; Prevelic, 2000; Abitbol & Abitbol, 2000; Amir & Biron-Shental, 2004; Lã, Ledger, Davidson, Howard & Jones, 2007; Lã, Howard, Ledger, Davidson & Jones, 2009). No studies have analysed intonation accuracy and vibrato rate and extent in singers, despite their complaints of difficulties controlling pitch and vibrato rate during the menstrual cycle.

Intonation involves at least three different types of simultaneously activated mechanisms (Larson et al., 1995; Mürbe, Pabst, Hofmann & Sundberg, 2002). One is preplanning and prephonatory tuning, to achieve the desired sound quality. It involves a number of different muscles, such as laryngeal, oropharyngeal and respiratory (Wyke, 1974). The second is auditory feedback, claimed to be an important tool for pitch control in singing (Burnet, Senner & Larson, 1997). It is used for matching the target pitch and to stabilize pitch production. Most people, particularly singers, use auditory feedback to make small adjustments when exposed to pitch shifted feedback (Burnett *et al.*, 1997). However, corrections of pitch errors based on auditory feedback are quite slow (about 200 ms, according to Grell, Sundberg, Ternström, Ptok & Atenmüller, 2009). The third mechanism is the kinaesthetic feedback, based on neuromuscular memory allowing laryngeal adjustments in response to pre-planning and auditory feedback. The accuracy of this feedback mechanism depends on the vocal task; it is poorest in fast and staccato singing (Mürbe et al., 2004). It can though be improved with vocal training (Mürbe, Pabst, Hofmann & Sundberg, 2002). Professional singers are able to sing with accurate pitch control even with masked auditory feedback (Schultz-Coulon, 1978; Mürbe et al., 2002, 2004).

Vibrato is another important characteristic of classical singing. Acoustically it corresponds to a low frequency modulation of the fundamental frequency (F0) at a rate of about 5 to 7 Hz, and the pitch perceived corresponds to the F0 mean (Sundberg, 1978;

Shonle & Horan, 1980). During short notes with vibrato however, the perceived pitch appears to be a function of the vibrato phase at the end of the note (d'Alessandro & Castellengo, 1994). If sinusoidal stimuli rather than sung sounds are used, up to three different pitches can be perceived (Van Besouw, Brereton & Howard, 2008). The F0 undulations are created by a pulsating contraction of the cricothyroid muscle and are thus produced by the neural system (Sundberg & Haglund, 1984). It is generally quite stable within a singer, although varying between the highest soprano notes (Bretos & Sundberg, 2003). Typically it decreases with age, on average by 0.25 Hz per decade (Sundberg, Niska-Thörnvik & Söderström, 1998).

As described above, several singers report intonation difficulties and vibrato changes accompanying the menstrual cycle. Changes in perceptual and motor skills have been found to be related to hormonal variations across the cycle (Hampson & Kimura, 1988; Sanders & Wenmoth, 1998; Saucier & Kimura, 1998). As hormones influence neural functioning, motor movement and sensory thresholds (Al-Mana, Ceranic, Djahanbakhc & Luxon, 2010), previous studies have hypothesised that continuous changes in the concentrations of sex steroid hormones interfere with the afferent and efferent processes involved in laryngeal neuromotor control (Higgins & Saxman, 1989; Isenberg, Brown & Rothman, 1983; Abramson et al., 1984). In addition, cyclical hormonal variations during the menstrual cycle have been reported to affect at least one temporal component of speech, namely voice onset time (VOT) (Whiteside, Hanson & Cowell, 2004).

Two hypotheses will be tested in the current investigation. One is that differences in intonation and in vibrato rate and extent can be observed between phases of the natural menstrual cycle. The second is that pitch control, is improved when using an OCP, as previous studies have found positive effects of OCP use on the periodicity of vocal fold

vibration (Amir et al., 2004; Lã et al., 2009). In this investigation the term pitch control will refer to the control of F0 of phonation.

METHOD

Study Design

The data were originally collected from co-author FL's doctoral dissertation (Lã et al., 2007; 2009). The initial aim was to assess the effects of an OCP on aspects of voice production in terms of periodicity of vocal fold vibration. The research design, extended over a total of 6 months, was a double blind randomised placebo controlled trial (RCT); both researchers and participants were unaware of the condition they were in (i.e. placebo or OCP). Thus, researchers and participants' expectations about this medication could not affect the outcome (Jadad, 1998).

The whole protocol and experimental design were approved by the South Sheffield Research Ethics Committee. As the experiment was not aimed to assess the effects of a new OCP, the risk was negligible; it was designed to test possible effects of a well tolerated OCP on the singing voice. There was, of course, the possibility that a participant may have fallen pregnant during the study; however, each participant was given precise information about the RCT and all agreed to use other forms of contraception during this period. A folder containing information regarding the details and purposes of the research was prepared and given to each singer.

Participants

Recruitment of reliable participants was necessary given the experimental design. Thus, a total of 17 female singers were recruited. An individual medical examination was carried out by a Professor in Gynaecology and Obstetrics, assessing each participant's suitability for the experiment. This guaranteed that each participant was suitable to use a

combined monophasic OCP which contained 30µg of ethinylestradiol and 3 mg of drospirenone. This particular OCP was chosen as it contains low-dosages of hormones, and is thus expected to cause fewer side effects and good cycle control (Huber *et al.* 2000; Foidart, 2000). During the consultation, details of the experimental design were explained to each participant, and identical numbered packs of OCP and matched placebo were given in a randomised order. Both pills were taken during 6 consecutive months, 3 for each condition (i.e. placebo or OCP). All participants had reported to have regular menstrual cycles. Thus, it was possible to predict approximately the date of the first recording and a recording calendar was made according to each participant's cycle.

All participants satisfied the inclusive criteria of being healthy, non-smokers, having regular menstrual cycles (raging from 28 to 30 days), and never having been or wishing to be pregnant at the time of the study. They were semi-professional western classically trained singers (mean age = 23.10 years, SD = 2.183; range = 21 to 27 years old), studying at several music colleges in the U.K.

Unfortunately, only 10 of the 17 singers managed to complete the RCT: three pulled out immediately before the first recording session; one became pregnant before starting the experiment; two completed only half of the RCT; and data collection for one participant could not be finished, because the singer moved out of the country. Additionally, one participant was excluded from the analysis because of problems with data collection. A total of 9 singers constituted the final data set. Eight were sopranos and 1 mezzo-soprano; five were using an OCP before the beginning of the experiment, and one of them was using already the OCP chosen for this study.

Procedure

For the current study, the OCP and matched placebo were randomly allocated in identical packaging by the Pharmacy Services Director of the Royal Hallamshire Hospital in Sheffield. Identical numbered packs of OCP and matched placebo were given in a randomised order to each participant at the end of the medical consultation. Therefore, neither the author, nor the participants was aware of the experiment conditions. Both pills were taken during 6 consecutive months, 3 for each condition (i.e. placebo or OCP). To monitor and ensure that the pills were taken correctly, a calendar of the days in which singers should take the pills or should have a break was provided to each participant.

Instructions were given to each participant concerning the use of OCP and matched placebo: the pills should be taken for 21 consecutive days, with a 7 day interval between packs. The study would start on the first day of the menstrual cycle, when the first pill of the 6 identical packs should be taken.

Data collection

For the third month of OCP and the third month of matched placebo intake, blood samples and audio-electrolaryngograh recordings were collected for each participant, at three points in the menstrual cycle, i.e. menstruation, and follicular and luteal phases. For the menstruation phase, data were collected on the second day of menses, for the follicular, on the 11^{th} day, and for the luteal, on the 24^{th} day of the cycle.

Blood samples were taken to measure concentrations of sex steroid hormones, i.e. oestradiol (E2, in pmol/l), progesterone (P, in nmol/l) and testosterone (T, in nmol/l), and to ensure a correct use of OCP and matched placebo. The IMMULITE® analyzer for *in vitro* was used to measure concentrations of E2 and P, and the ADVIA Centaur™ System for *in*

vitro was used to measure concentrations of T in serum. Calculations of estrogens/progesterone ratio (E2/P) were also made.

Voice recordings were carried out using an omnidirectional condenser microphone with a flat response within 2 db between 20 and 20 000Hz, connected to a microphone preamplifier, a two channel stereo digital audio tape-recorder, and to a portable Electrolaryngograph (Laryngograph Ltd.) with an oscilloscope incorporated, thus facilitating optimal electrode placement.

Participants were asked to sing a vocal exercise that consisted of an ascending octave, followed by a descending major triad using the vowel [a] (see Figure 1). This particular exercise was chosen as it is commonly used for voice warm-up. In this way, the risk of effects on intonation inaccuracy due to unfamiliarity with the vocal task was minimised. In addition, this exercise contains an ascending octave interval aiming at a sustained note at the top; thus, reliable F0 extraction could be made, facilitating accurate measurement of interval size and vibrato rate and extent. It also seemed reasonable to assume that a large interval, such as the octave, would more clearly expose potential intonation difficulties than a narrower interval.

(please insert Figure 1 about here)

Voice analysis

From the recordings, samples of this singing exercise starting at pitches F4 and B4 were selected for analysis. These particular pitches were chosen as they are: (i) close to a register transition (pitch F5), and (ii) close to the upper limit of a soprano's range (pitch B5) (Titze, 2000).

This material was analyzed for the three phases of the menstrual cycle during the third month for both placebo and OCP conditions. This yielded a total of 108 renderings (9 subjects x 2 pitches x 3 phases and x 2 conditions).

The selected recorded files were analysed using the Soundswell signal workstation. For each participant these samples were transferred to an analysis file. The pitch tracking was carried out by means of autocorrelation (the *Corr* tool of the Soundswell software Granqvist & Hammarberg, 2003) applied to the audio or the electrolaryngograph signal (ELG), depending on what signal gave the most reliable output. The first and second tones were selected for analysis as they were long and reasonably stable, while the remaining tones were often quite short and unclearly represented in the F0 curves. The ascending octave between the first and second tones also seemed to be the target of this exercise (see example provided in Figure 2). The *Histogram* tool of the Soundswell signal workstation was used to determine the mean F0 values of these two tones. The values obtained were then transferred to an excel file where the interval between them was calculated in semitones.

(please insert Figure 2 about here)

Voice parameters

As some participants tended to consistently produce wide or narrow octaves, the signed deviation from pure octave (SgD, in semitones) was calculated. It represents how sharp or flat the octave was as compared to the frequency ratio of 2:1. In addition, vibrato rate (VbR, in Hz) and extent (VbEt, in cent, i.e., hundredths of a semitone) were measured for the sustained top note of the octave intervals. The mean F0 of the vibrato curve was first calculated followed by the identification of where the rising parts of the vibrato curve crossed the mean F0, and then measuring the time intervals between these crossings (see

Figure 3). The inverted mean of these intervals was considered as the mean vibrato rate. An alternative method was to display the Laryngograph signal as a narrow band spectrogram in which the peaks and valleys of a clearly visible high frequency partial were measured manually. Then, the data were transferred to an excel file, where the mean rate and the mean extent were calculated.

(please insert Figure 3 about here)

Statistical analysis

Due to the small sample size and since each participant was evaluated under all conditions (repeated-measures design), robust descriptive statistics (median and interquartile range) and non-parametric k-related samples hypothesis test based on rank comparisons (Friedman and Wilcoxon's Signed Rank tests) were calculated. The level of significance used was $\alpha = 0.05$. Friedman test was used to examine whether there was a significant difference between the three phases of the cycle within each condition (i.e. placebo or OCP). Wilcoxon's Signed Rank test was applied to test whether there were significant differences between placebo and OCP use for each of the threes phases of the cycle. Since this test involves three simultaneous comparisons, a Bonferroni correction was considered and so, these results were identified as significant when $p<0.05/3 = 0.017$.

Boxplots were drawn to display differences between phases within the same cycle for each condition, and also to display differences between placebo and OCP use. This type of graphical representation allows the identification of possible moderate (○) and severe outliers (*) in the data. All statistical analyses were conducted using the SPSS 17.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Sex Steroid Hormones

Table 1 shows the median (Med) and interquartile range (IQR, equal to difference between the third and first quartiles) for concentrations of sex steroid hormones, for both placebo and OCP use, during the three phases of the menstrual cycle. Descriptive results in terms of means and standard deviations for these concentrations were previously published (Lã et al., 2007). However, as non-parametric statistical tests were used for data analysis, median and interquartile range were selected for descriptive statistics to calculate all the presented results (median, as a measure of central tendency representing the 50th percentile, and interquartile range, as a measure of variability representing the difference between 75th percentile and 25th percentile).

(please insert Table 1 about here)

Oestrogen concentrations (measured as oestradiol) were significantly different between the three phases only for the placebo condition, being lower during the menstrual phase and higher during the luteal phase (Placebo: $\chi^2(2) = 10.889$, $p = 0.003$; OCP: $\chi^2(2) = 0.706$, $p = 0.758$) (see Figure 4). When comparing conditions, significant differences were found observing lower concentrations in the luteal phase for OCP use (menstrual phase: $z = -2.073$, $p = 0.039$; follicular phase: $z = -1.955$, $p = 0.055$; luteal phase: $z = -2.666$, $p = 0.004$) (see Figure 4).

Progesterone concentrations were significantly different between the three phases of the cycle, for the placebo and OCP use (Placebo: $\chi^2(2) = 13.556$, $p < 0.001$; OCP: $\chi^2(2) = 7.600$, $p = 0.020$). Significant difference was found between conditions caused by the luteal phase (menstrual phase: $z = -2.033$, $p = 0.047$; follicular phase: $z = -1.724$, $p = 0.102$; luteal phase: $z = -2.666$, $p = 0.004$). Clearly higher concentrations were observed for the luteal phase during placebo use (see Figure 4).

Significant differences were found for testosterone concentrations between the three phases of the cycle only for the placebo condition (Placebo: $\chi^2(2) = 7.371$, $p = 0.023$; OCP: $\chi^2(2) = 3.000$, $p = 0.285$). However, concerning differences between conditions, placebo and OCP use displayed significantly different concentrations of testosterone for the follicular phase of the cycle (menstrual phase: $z = -1.970$, $p = 0.063$; follicular phase: $z = -2.386$, $p = 0.016$; luteal phase: $z = -0.980$, $p = 0.383$), OCP showing lower concentrations as compared to the placebo (see Figure 4).

When assessing whether there were differences for the E2/P ratio between the three phases within each condition, differences were found only for the placebo use (Placebo: $\chi^2(2) = 11.556$, $p = 0.001$; OCP: $\chi^2(2) = 3.556$, $p = 0.187$). E2/P ratio was significantly different between placebo and OCP conditions, this being due to the lower concentrations for the follicular phase during OCP use (menstrual phase: $z = -0.178$, $p = 0.910$; follicular phase: $z = -2.666$; $p = 0.004$; luteal phase: $z = -2.310$, $p = 0.020$) (see Figure 4).

(please insert Figure 4 about here)

Vocal parameters

Figures 5 to 7 display the SgD, VbR and VbEt data, for each phase of the cycle, for each condition, for each participant and for the two pitches. The variations between participants are quite substantial but appear unrelated to voice classification. Thus, the results from the mezzo-soprano (participant 9) did not deviate from those of the other singers. Regarding VbEt, a great difference can be observed between the two pitches, B5 showing quite low values. Two singers could not complete their tasks (VbR and VbEt) for pitch B5 and were excluded from analysis.

(please insert Figures 5, 6, and 7 about here)

Table 2 shows the corresponding medians and interquartile ranges for the analysed vocal parameters (SgD, VbR, and VbEt).

(please insert Table 2 about here)

Comparing SgD for the F5 pitch, significant differences were observed between phases only for OCP use (Placebo: $\chi^2(2) = 2.000$, $p = 0.398$; OCP: $\chi^2(2) = 8.00$, $p = 0.019$), the follicular phase presenting the lowest values. No significant differences were found between placebo and OCP use for any of the phases for this pitch (menstrual phase: $z = -0.889$, $p = 0.426$; follicular phase: $z = -2.192$, $p = 0.027$; luteal phase: $z = -0.178$, $p = 0.910$).

Still looking at pitch F5 but now regarding VbR, no significant differences were found between phases within each condition (Placebo: $\chi^2(2) = 1.086$, $p = 0.654$; OCP: $\chi^2(2) = 5.556$, $p = 0.069$). However, significant differences were found between placebo and OCP use for the follicular phase (menstrual phase: $z = -1.718$, $p = 0.098$; follicular phase: $z = -2.547$, $p = 0.008$; luteal phase: $z = -1.244$, $p = 0.250$).

For VbEt for the F5 pitch, no significant differences were observed between phases within each condition (Placebo: $\chi^2(2) = 4.222$, $p = 0.154$; OCP: $\chi^2(2) = 0.889$, $p = 0.685$). Additionally, there were no significant differences between placebo and OCP use for any of the menstrual phases (menstrual phase: $z = -0.059$, $p = 1.000$; follicular phase: $z = -1.599$, $p = 0.129$; luteal phase: $z = -1.599$, $p = 0.129$).

For the higher pitch no significant differences were observed for SgD between phases within each condition (Placebo: $\chi^2(2) = 2.000$, $p = 0.398$; OCP: $\chi^2(2) = 0.222$, $p = 0.971$). Additionally, significant differences were not found between placebo and OCP use for any of the menstrual phases (menstrual phase: $z = -1.481$, $p = 0.164$; follicular phase: $z = -1.244$, $p = 0.250$; luteal phase: $z = -0.059$, $p = 1.000$).

Also for VbR, no significant differences were found between phases within each condition for pitch B5 (Placebo: $\chi^2(2) = 5.154$, $p = 0.076$; OCP: $\chi^2(2) = 2.154$, $p = 0.384$). Additionally, no significant differences were found between placebo and OCP use for any of the menstrual phases (menstrual phase: $z = -2.120$, $p = 0.047$; follicular phase: $z = -0.424$, $p = 0.703$; luteal phase: $z = -0.775$, $p = 0.469$).

Finally, results also show no statistically significant differences for VbEt between phases within each condition (Placebo: $\chi^2(2) = 2.571$, $p = 0.305$; OCP: $\chi^2(2) = 0.000$, $p = 1.000$). No significant differences were found between placebo and OCP use for any menstrual phase for this pitch (menstrual phase: $z = -0.676$, $p = 0.576$; follicular phase: $z = -0.338$, $p = 0.813$; luteal phase: $z = -0.676$, $p = 0.578$).

A summary of these statistical results is depicted in Table 3 and Figure 8: significant statistically differences were observed in octave size only for the F5 pitch. These differences were found between the three phases of the menstrual cycle for the OCP condition only: the deviation from pure octave was narrower in the follicular phase, in terms of the median (see Figure 8). When phases of the cycle were compared between conditions, significant differences were found only for VbR at the F5 pitch for the follicular phase: the vibrato rate was lower during OCP use than during placebo use (see Figure 8).

(please insert Table 3 and Figure 8 about here)

DISCUSSION

Comparisons between the three phases for the placebo condition revealed significant variations for all sex steroid hormones (i.e. oestrogens, progesterone, testosterone and E2/P ratio). However, for the OCP condition, differences between phases were found only with respect to progesterone concentrations. Thus, the expected stabilizing effect of OCP use on hormonal variations across the menstrual cycle was confirmed

(Speroff, Glass & Kase, 1989). Comparisons between placebo and OCP conditions for each phase showed: (i) significantly reduced concentrations of both oestrogens and progesterone for the luteal phase during OCP use; and (ii) significantly reduced E2/P ratio and concentrations of testosterone for the follicular phase also for OCP use, the latter confirming antiandrogenic effects of this particular OCP (Huber et al., 1965; Foidart, 2000).

Given the results of previous studies as well as several singers' reports, one would expect that the constant variations in sex steroid hormones during placebo use would lead to significant changes in pitch control across the three phases of the cycle (Isenberg, Brown & Rothman, 1983; Lã & Davidson, 2005). However, this expectation was not statistically evidenced from the data set. Variations in hormonal concentrations across the cycle during placebo use seemed not to be reflected in pitch intonation and vibrato rate and extent; no significant differences between the three phases were found for these vocal parameters.

Taking into account the dampening of hormonal variations provided by OCP use, an improvement in intonation accuracy and vibrato rate and extent control was expected. Once again, this was not confirmed by the results. Instead, data showed significant differences in intonation between the three phases of the cycle only during OCP use and only for the F5 pitch. The octave was narrower during the follicular phase than during the other two phases. Moreover, when looking at VbR, differences between placebo and OCP use were found concerning the follicular phase of the cycle, and once again only for the F5 pitch. The vibrato rate was slowed down during OCP use: for the follicular phase VbR was significantly lower in the OCP condition ($Med_{VbR} = 4.79$) than in the placebo condition ($Med_{VbR} = 5.50$). As previous studies suggest that hormones influence neural functioning, motor movement and sensory thresholds (Al-Mana et al., 2010), one would expect that also

VbEt would be affected by changes in the concentrations of sex steroid hormones. However, no VbEt effects were observed under any condition.

In view of the above, the most striking outcome of this investigation seems to be the complex interactions between hormonal milieu and aspects of pitch control. In fact, one can argue that more questions were raised than answered. Why could effects on octave size and VbR be found only for the F5 pitch and not for B5? What could explain the significant differences in octave size between the three phases only during OCP use? What factors could account for the significant differences found in VbR for the follicular phase between placebo and OCP use? This multitude of questions illustrates the complexity underlying the observed effects. Thus, only speculations can be presented regarding factors that could account for each of the findings.

As effects on interval size and vibrato rate were observed only for the F5 pitch, one could speculate that hormonal effects on pitch control are rather mild and may have been over shadowed by inter/intra individual variation. Perhaps these effects are manifested only for notes that require exceptional vocal control, such as those within the *passaggio* region. Physiologically, difficulties with *passagio* tones reflect the need for extremely precise adjustments of thyroarytenoid and cricothyroid activation in combination with fine tuning of subglottal pressure (Titze, 2000; Thurman, Welch, Feit & Grefsheim, 2000). It is possible that even slight disturbances of the system controlling these adjustments, such as those related to sex steroid hormones and OCP use, might be sufficient to impact on pitch control within but not outside the *passaggio* range. Most vocal pedagogues agree that many singers fail to produce the B5 pitch with a vibrato. Hence, this might have accounted for the small VbEt on this pitch among participants.

The second question raised was what could explain the significant differences in octave size between the three phases only during OCP use? A factor that very well may have contributed to the modifications of the octave size is changes in the accuracy of fundamental frequency (F0) production. F0 depends on two factors, physical properties of the vocal folds and neural/auditory control.

The mechanical properties of the vocal folds might change according to hormonal variations (Abitbol, Abitbol & Abitbol, 1999; Amir & Biron-Shental, 2004; Lã et al., 2007). For example, increased concentrations of oestrogens have been related to an increase in the secretion of the glandular cells above and below the vocal fold edges, which would modify the mucosa of the vocal folds and thus lead to minor changes in voice production (Abiltbol et al., 1999). However, more significant voice changes have been associated with changing concentrations of progesterone and testosterone. Progesterone increases the viscosity and acidity of the secretions of the glandular cells, but decreases their volume, causing a relative dryness (Abitbol et al., 1999), which has been found to affect phonation pressure threshold, thus suggesting an effect on vocal fold motility (Verdolini-Marston, Finkelhor, Titze & Durham, 1998). The properties of the vocal folds may be changed under the influence of testosterone or other androgens, since these hormones might increase the amount of water in the ground substance, thus compromising the extensibility, range and steadiness of the voice (Baker, 1999). In the present investigation, both testosterone and E2/P ratio were significantly lowered during OCP than for placebo use for the follicular phase, when also significant differences in VbR and almost significant effect on intonation ($p = 0.027$) were found. It is possible that hormonal effects on pitch control result from a specific combination of concentrations of different hormones rather than from the effects of specific hormonal concentrations. This has also been suggested by other studies assessing

the effects of steroid hormones on the auditory system (Al-Mana et al., 2010) and voice production (Abitbol et al., 1999).

With regard to neural control of F0, changes in the concentrations of sex steroid hormones across the natural menstrual cycle have been associated with interferences with the laryngeal afferent and efferent neuromotor control (Higgins & Saxman, 1989; Isenberg et al., 1983; Abramson et al., 1984). Additionally, voice onset time (VOT) was showed to be affected by cyclical hormonal variations during the menstrual cycle (Whiteside et al., 2004). The results of our study concerning with the three vocal parameters SgD, VbR and VbE do not corroborate these findings, as no differences were found between the phases during placebo use. During OCP use, there was a significant phase difference only with respect to progesterone concentrations. It is interesting to note that this difference was accompanied by a difference in the octave size for the pitch of F5. On the other hand, no corresponding effect was observed to accompany the much greater hormonal variations between phases during placebo use. Once again, this supports the assumption that intonation is dependent on several concomitant factors.

As mentioned in the introduction, intonation is dependent on the concomitant recruitment of a number of different mechanisms: prephonatory, kinaesthetic and auditory (Larson et al., 1995; Mürbe et al., 2002). Of these, at least the auditory feedback mechanism is affected by hormonal variations (Walpurger, Pietrowskyb & Kirschbaumc, 2004; Al-Mana, Ceranic, Djahanbakhc & Luxon, 2008; Al-Mana et al., 2010); oestrogen receptors have been found in the cochlea (Stenberg, Heimer, Holmberg & Ulmsten, 1999; Charitidi, Meltser, Tahera & Canlon, 2009) and progesterone was found to interact with other steroid binding sites present in the auditory system (Majewska, Harrison, Schwartz, Barker & Paul, 1986; Follesa et al., 2001). Mild effects of sex steroid hormones on auditory

functioning have been found during the ovarian cycle (Al-Mana et al., 2010). High concentrations of oestrogens seem to have an excitatory effect facilitating auditory information (Charitidi et al., 2009), whereas high concentrations of progesterone counterbalance these effects (Katzenellenbogen, 2000; Smith, Adams, Schmidt, Rubinow & Wassermann, 2002) and may negatively impact on the ear and central auditory system (Guimarães et al., 2006; Price, Guimarães, Vasilyeva & Frisina, 2009). Thus, previous studies suggest complex effects on the auditory system as a result of multifaceted interactions between oestrogens and progesterone (Al-Mana et al., 2010). Hence, it seems fair to speculate that the combination of lower E2/P ratio and reduced testosterone during OCP use could have contributed to the significant effect on the octave size.

The VbR values observed were marginally lower than those observed by Bretos and Sundberg (2003) for the pitches F5 and A5 in commercial recordings of ten sopranos. These lower VbR values, observed during the follicular phase for the OCP condition, may reflect effects of OCP use on the neural control system. Previous studies have found a slowing down of the speed of neural transmission, phenomena associated with reductions of both ovarian hormones and testosterone during OCP use (Caruso et al., 2003). VbR is likely to depend on the rate of neural transmission. If so, the reduction of concentrations of testosterone and E2/P ratio found in our study during OCP use could have caused the slowing down of the speed of neural transmission and hence also VbR.

Effects on vibrato extent were found for none of the notes, neither for the octave size for the higher note. The multi-factorial nature of the body responses to the endocrine system as well as its complexity may very well have concealed the effects of OCP use and hormonal variations. Moreover, singers, like other highly skilled athletes, are trained to

perform under non-optimal physical and mental conditions, thus possibly hiding hormonal effects on their voice control.

Finally, sex steroid hormonal variations have been found to impact on: vocal fold vibratory patterns (Amir et al., 2004; Lã et al., 2009); auditory function (Walpurger et al., 2004; Al-Mana et al., 2008); cognitive function (Hampson, 1990); neural excitability (Smith et al., 2002); and some sensorial processes (Eisner, Burke & Toomey, 2004; Giuffre, Di Rosa & Fiorino, 2007; Grillo et al., 2001). It is possible that interactions between all these systems could concomitantly affect pitch control and vibrato rate during OCP use for pitches demanding exceptional vocal control, such as *passaggio* notes.

Some participants showed great variations during the cycle, which is in accordance with reported experiences of many singers. For example, during placebo use, subject 3 was producing a quite narrow octave on F5 in the menstrual phase but clearly wider octaves during the two other phases; also on B5 her octave size varied substantially between phases, as was illustrated in Figure 5. This is in accordance with many singers' experiences of variations of voice function in synchrony with the phases of the menstrual cycle. A complementary, longitudinal study of such singers may substantiate such reports.

CONCLUSIONS

For pitches within the *passagio* range, singers performed a narrower octave interval and sustained notes with a slower vibrato rate during OCP than during a natural menstrual cycle. It is possible that these effects were due to concomitant impacts of sex steroid hormones on vocal fold vibratory patterns, auditory and cognitive functions, and neural excitability.

Corresponding effects could not be observed neither when singers performed a higher note, nor with respect to vibrato extent. The multi-factorial nature of bodily responses to the endocrine system as well as the singer's ability to perform under non-optimal physical and mental conditions may very well have concealed additional effects of OCP use and sex steroid hormonal variations. It would be worthwhile in the future to analyse the impacts of sex steroid hormonal on singers' performances taking into account the underlying complexity of the endocrinology of the voice.

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Table 1: Summary results of the statistical tests carried out for hormonal concentrations during the three phases of the menstrual cycle and for the two conditions (n=9). $^{+}$ Friedman test, statistical significance ($p<0.05$). ‡ Wilcoxon's Sign Rank test, statistical significance ($p<0.017$). E2: Oestradiol (pmol/l); P: progesterone (mmol/l); T: testosterone (mmol/l); E2/P ratio: ratio between oestradiol and progesterone. Med: Median; IQR: Interquartile range.

	Menstrual phase				Follicular phase				Luteal phase			
	Placebo		OCP		Placebo		OCP		Placebo		OCP	
Hormonal	Med	IQR	Med	IQR	Med	IQR	Med	IQR	Med	IQR	Med	IQR
E2	130.00^{+}	70.50	82.00	64.60	196.00^{+}	360.50	73.00	50.55	437.00$^{+‡}$	350.50	56.9‡	15.60
P	3.00^{+}	2.20	1.80^{+}	1.85	2.50^{+}	2.10	3.30^{+}	4.65	30.70$^{+‡}$	22.55	2.10$^{+‡}$	1.45
T	1.50^{+}	0.90	1.20	0.80	1.80$^{+‡}$	0.80	1.10‡	0.70	1.70^{+}	1.15	0.90	1.15
E2/P ratio	40.43^{+}	27.37	36.96	47.74	64.64$^{+‡}$	272.29	22.12‡	22.78	14.42^{+}	8.18	28.68	14.26

Table 2: Summary results of the statistical tests carried out for vocal parameters for the two pitches (i.e. F5 and B5), during the three phases of the menstrual cycle and for the two conditions. [+] Friedman test, statistical significance ($p<0.05$). [‡] Wilcoxon's Sign Rank test, statistical significance ($p<0.017$). SgD: Signed deviation from pure octave (in semitones); VbR: vibrato rate (Hz); VbEt: vibrato extent (cent). Med: Median; IQR: Interquartile range.

Vocal parameters		Menstrual phase				Follicular phase				Luteal phase			
		Placebo		OCP		Placebo		OCP		Placebo		OCP	
		Med	IQR	Med	IQR	Med	IQR	Med	IQR	Med	IQR	Med	IQR
F5	SgD (n=9)	0.12	0.68	0.14[+]	0.48	0.18	0.74	-0.15[+]	0.79	-0.09	0.48	0.03[+]	0.96
	VbR (n=9)	5.24	1.63	6.49	1.58	5.50[‡]	0.97	4.79[‡]	0.72	5.21	1.49	5.40	1.65
	VbEt (n=9)	158.94	147.66	192.85	116.52	190.03	89.97	266.39	233.58	145.94	153.09	193.66	128.58
B5	SgD (n=9)	-0.17	0.45	0.16	0.76	0.05	0.5	0.16	0.39	0.03	0.38	0.01	0.29
	VbR (n=7)	5.30	2.40	5.20	2.60	5.00	1.90	5.90	2.70	5.00	2.20	5.10	3.00
	VbEt (n=7)	81.00	109.00	94.00	108.00	70.00	154.00	97.00	82.00	60.00	91.00	87.00	88.00

Table 3: Summary results of the statistical tests carried out for concentrations of sex steroid hormones and for analysed vocal parameters of the two pitches considered (i.e. B5 and F5). A Friedman test was carried out to evaluate if there are significant statistically differences ($p<0.05$) between the three phases of the menstrual cycle within each condition (i.e. placebo or OCP), while a Wilcoxon's Sign Rank test was conducted to assess whether there are significant statistically differences ($p<0.017$) between conditions (i.e. placebo and OCP) for each phase of the menstrual cycle. E2: Oestrogen; P: progesterone; T: testosterone; E2/P ratio: ratio between oestrogen and progesterone. SgD: Signed deviation from pure octave (in semitones); VbR: vibrato rate (Hz); VbEt: vibrato extent (cent). M: menstrual; F: follicular; L:Luteal.

Hypothesis		Concentrations of sex steroid hormones				F5			B5			Test
		E2	P	T	E2/P	SgD	VbR	VbEt	SgD	VbR	VbEt	
H0: M=F=L	Placebo	0.003^{+}	$<0.001^{+}$	0.023^{+}	0.001^{+}	0.398	0.654	0.154	0.398	0.076	0.305	Friedman $p<0.05^{+}$
H0: M=F=L	OCP	0.758	0.020^{+}	0.285	0.187	0.019^{+}	0.069	0.685	0.971	0.384	1.000	
H0:Placebo=OCP	M	0.039	0.047	0.063	0.910	0.426	0.098	1.000	0.164	0.047	0.576	Wilcoxon's Sign Rank $p<0.017^{‡}$
	F	0.055	0.102	$0.016^{‡}$	$0.004^{‡}$	0.027	$0.008^{‡}$	0.129	0.250	0.703	0.813	
	L	$0.004^{‡}$	$0.004^{‡}$	0.383	0.020	0.910	0.250	0.129	1.000	0.469	0.578	

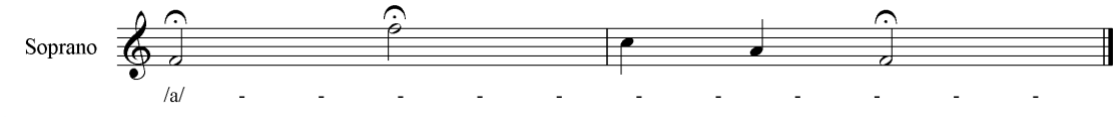


Figure 1: The vocal exercise used, here starting at pitch F4.

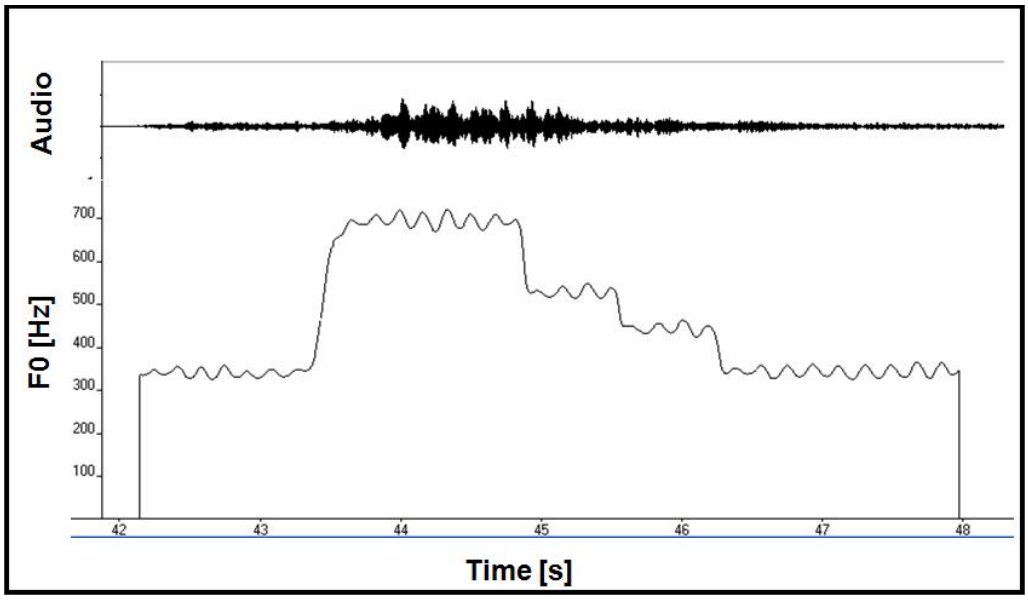


Figure 2: Illustration of the method used for F0 extraction of the ascending octave.

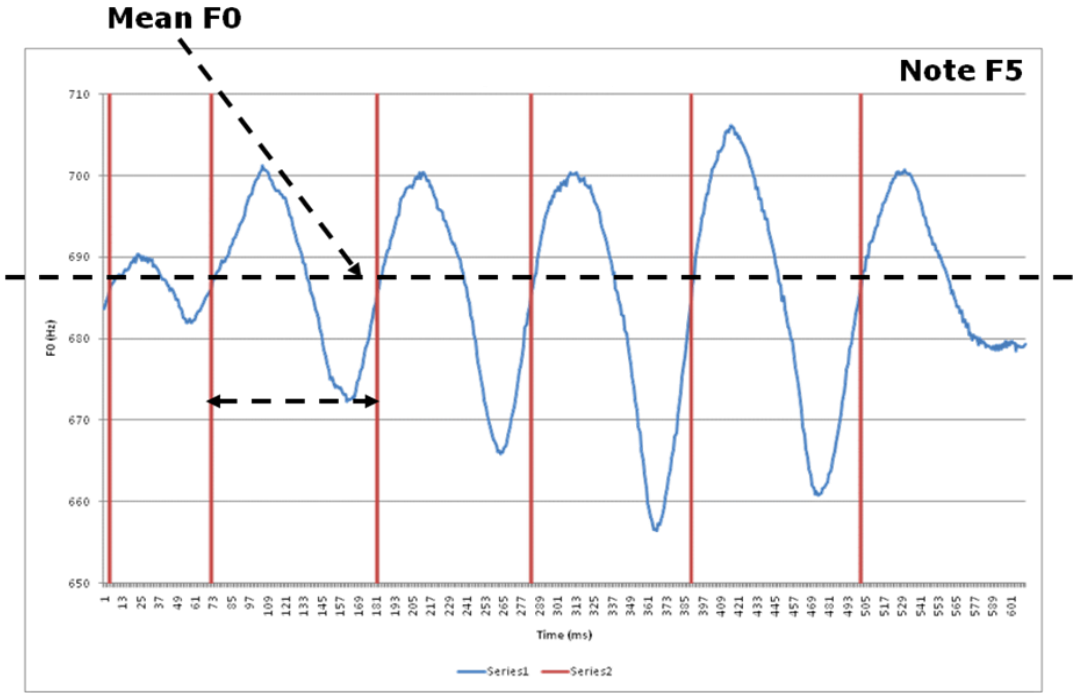


Figure 3: Illustration of the method used to extract both vibrato rate and extent.

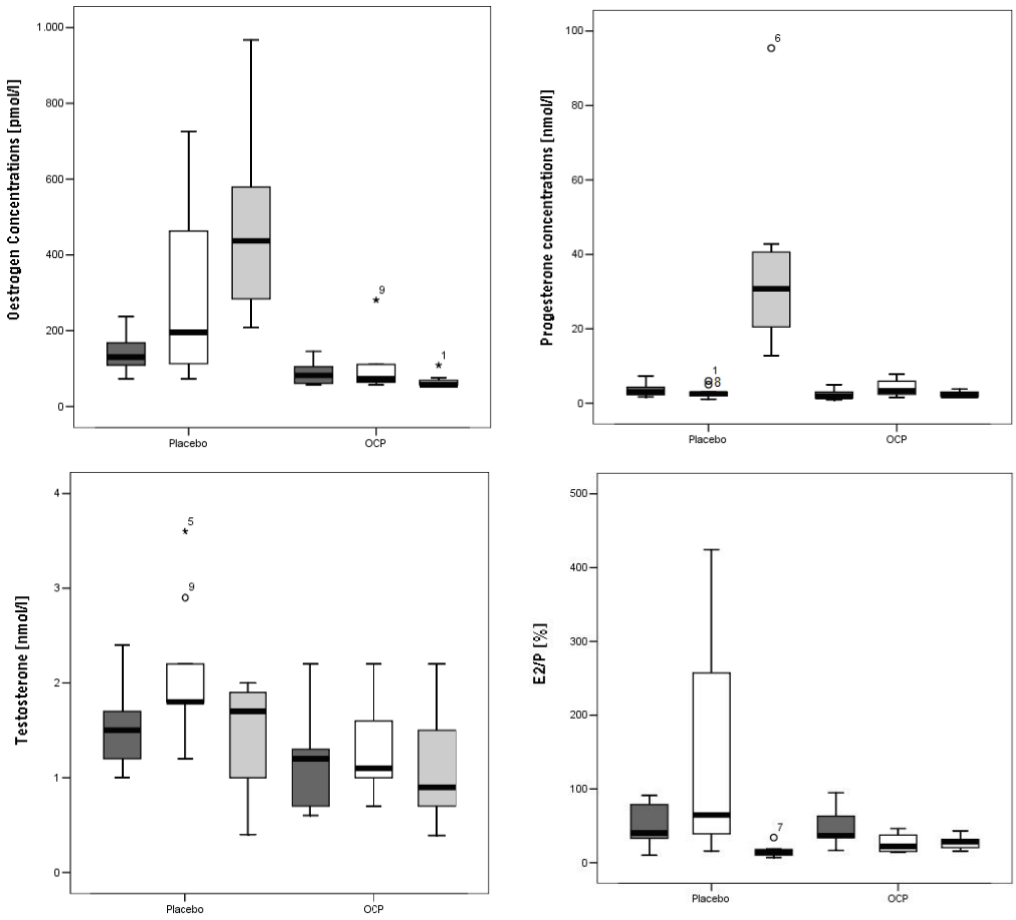


Figure 4: Concentrations of oestrogen (in pmol/l, top left), progesterone (in nmol/l, top right), testosterone (in nmol/l, bottom left), and oestrogens/progesterone ratio (bottom right) during placebo and OCP use, for the three phases of the menstrual cycle (n=9). Dark gray colour corresponds to the menstrual phase; White colour corresponds to the follicular phase; Light gray corresponds to the luteal phase. For the concentration of oestrogen, singers nº9 and nº1 are considered severe outliers for OCP use, during the follicular and luteal phases, respectively. For the concentration of progesterone, singers nº1, 8, and nº6 are considered moderate outliers for placebo use, during follicular and luteal phases, respectively. For the concentration of testosterone, singers nº9 and 5 are considered moderate and severe outliers, respectively, for placebo use during the follicular phase. For the oestrogens/progesterone ratio, singer nº7 is considered a moderate outlier for placebo use during the luteal phase.

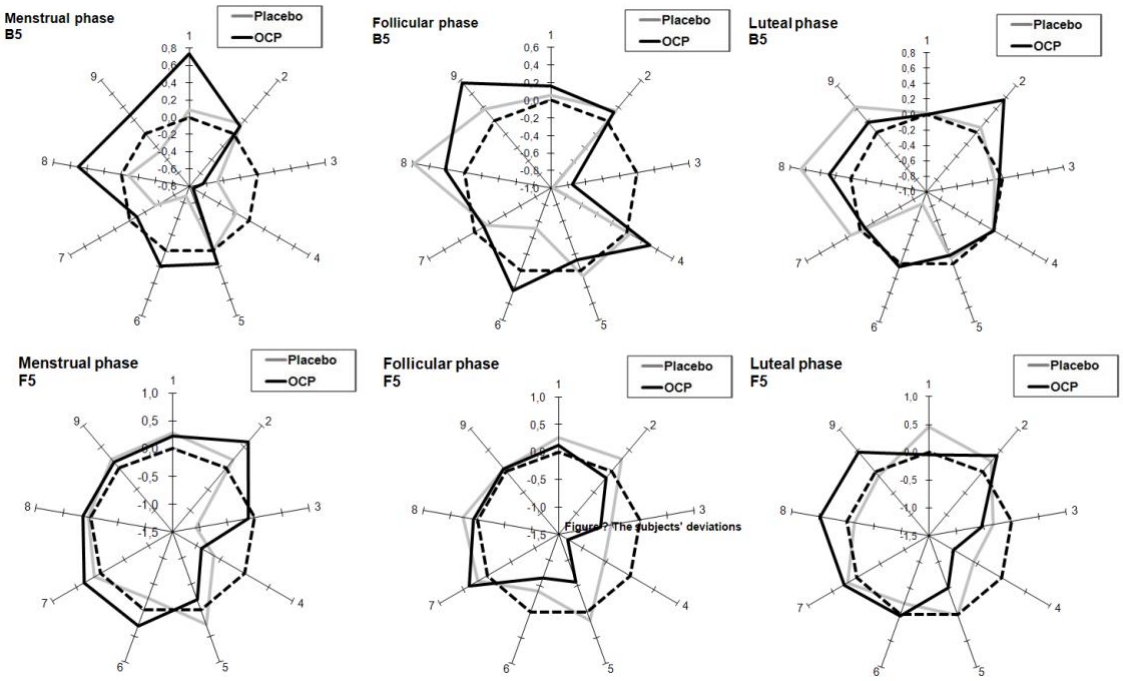


Figure 5: Participants' signed deviations (in semitones) from pure octave for the indicated pitches (F5 and B5) and for the three phases of the cycle (left, middle and right panels). The gray and black solid curves refer to the placebo and OCP conditions, respectively, and the dotted curves to the 2:1 interval size.

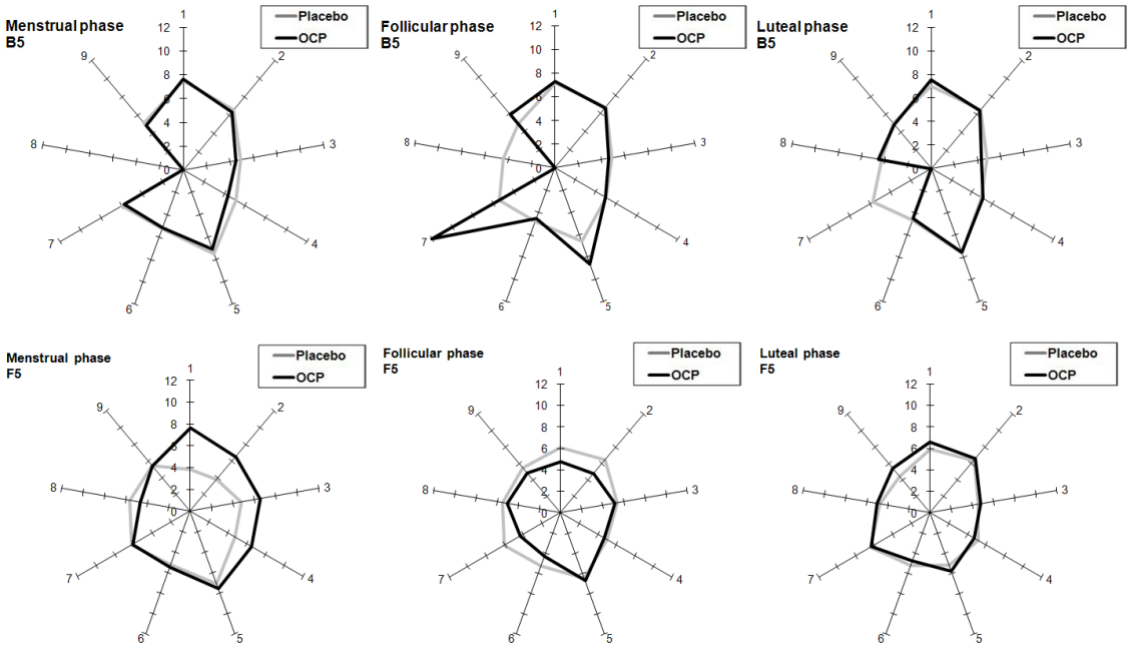


Figure 6: Participants' vibrato rates (in Hz) for the indicated pitches (F5 and B5) and for the three phases of the cycle (left, middle and right panels). The gray and black curves refer to the placebo and OCP conditions, respectively.

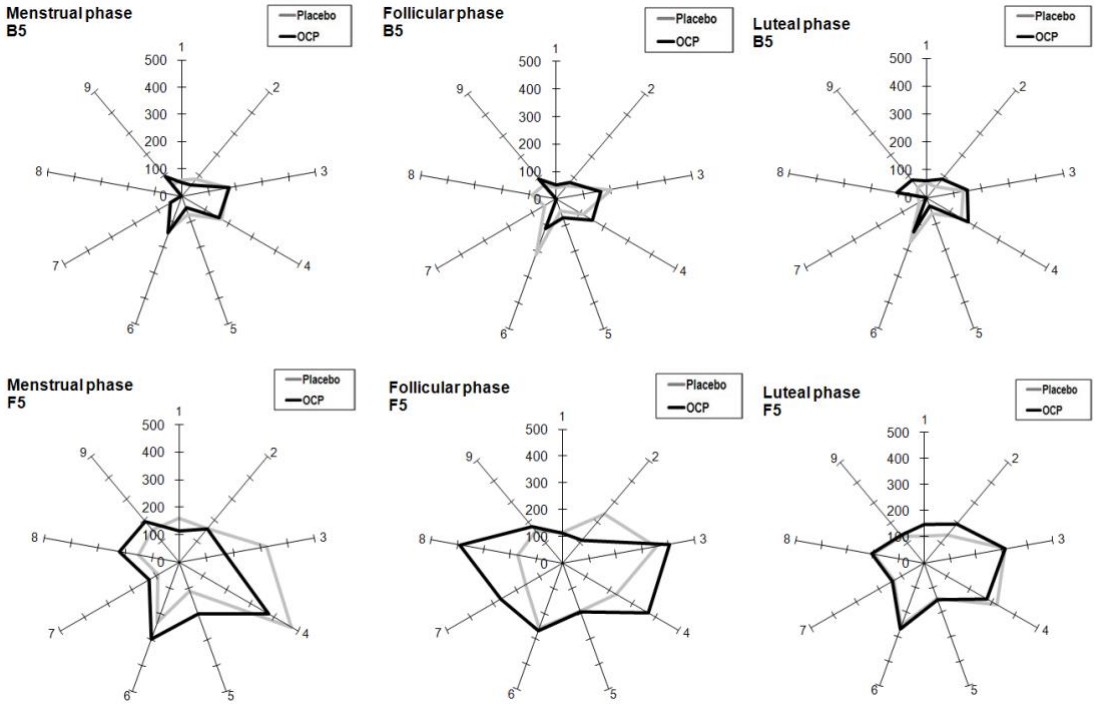
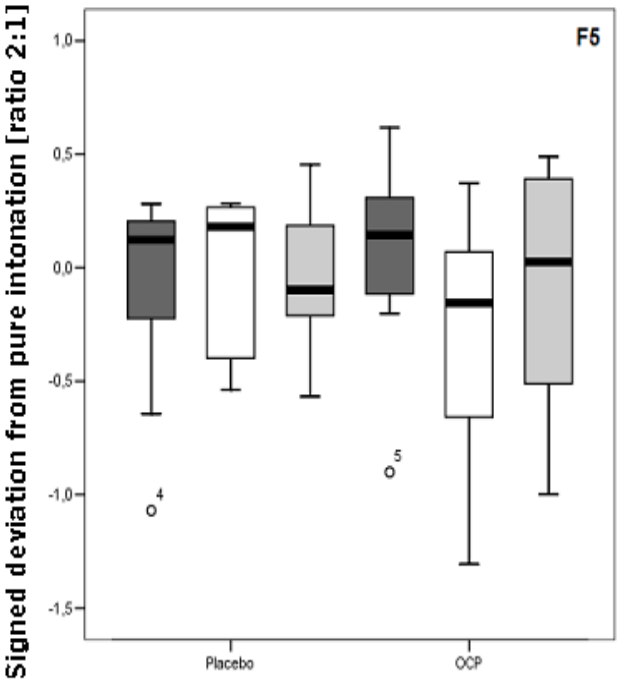
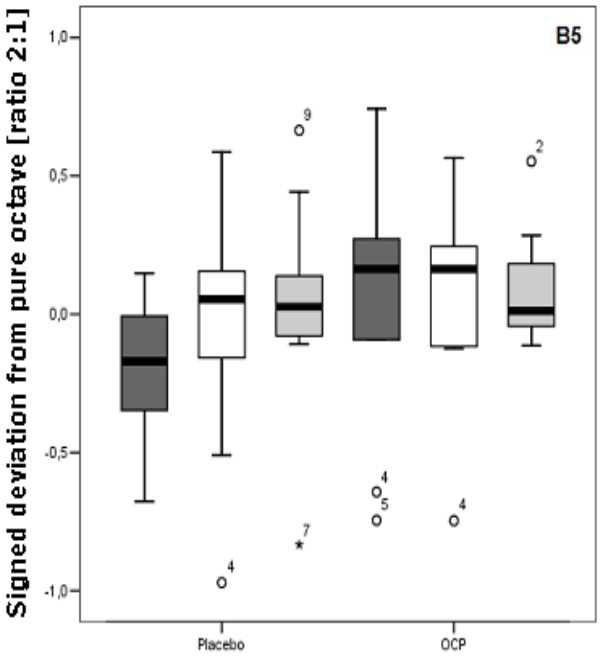
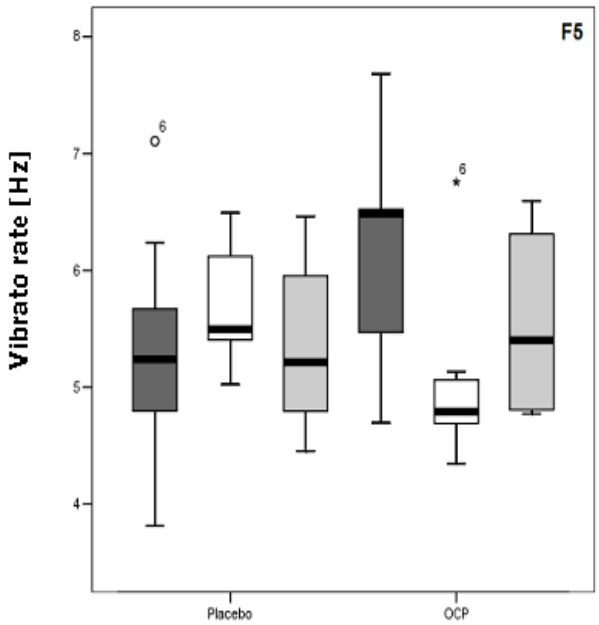
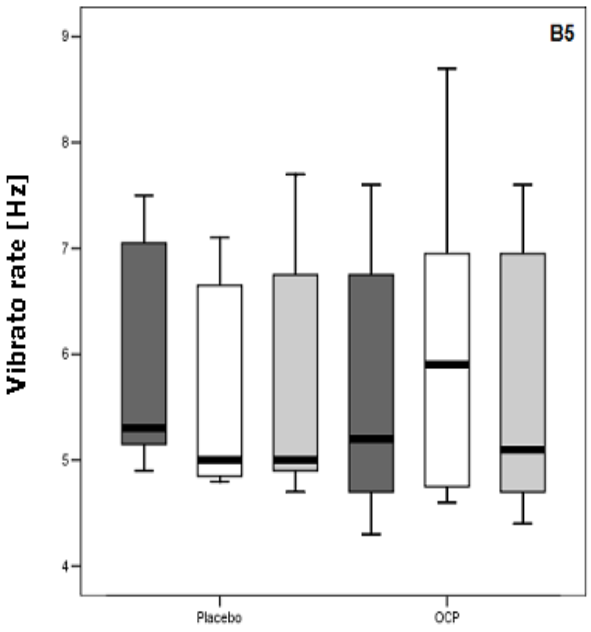


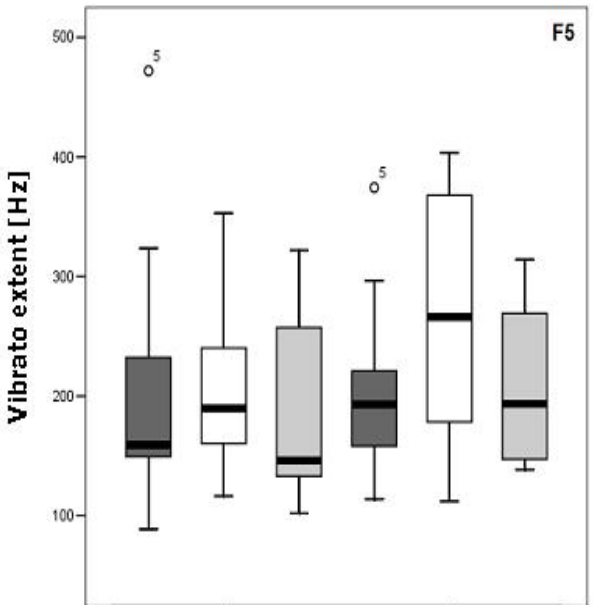
Figure 7: Participants' vibrato extents (in cent) for the indicated pitches (F5 and B5) and for the three phases of the cycle (left, middle and right panels). The gray and black curves refer to the placebo and OCP conditions, respectively.


F5
Signed deviation from pure intonation [ratio 2:1]
1,0
0,5
0,0
-0,5
-1,0
-1,5
Placebo
OCP


B5
Signed deviation from pure octave [ratio 2:1]
1,0
0,5
0,0
-0,5
-1,0
Placebo
OCP


F5
Vibrato rate [Hz]
8
7
6
5
4
Placebo
OCP


B5
Vibrato rate [Hz]
9
8
7
6
5
4
Placebo
OCP


F5
Vibrato extent [Hz]
500
400
300
200
100

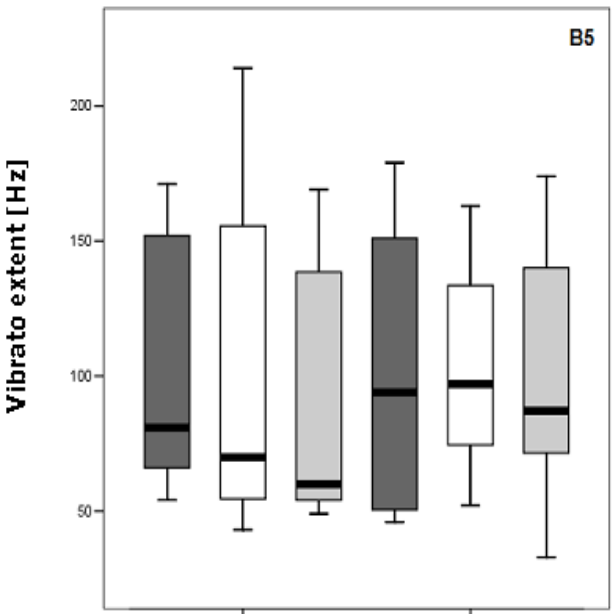

B5
Vibrato extent [Hz]
200
150
100
50

Figure 8: Signed deviation from pure octave (top panel, SgD), vibrato extent (middle panel, VbEt in Hz), and Vibrato rate (bottom panel, VbR in Hz) during Placebo and OCP use, for the three phases of the menstrual cycle and the two pitches B5 (left panels) and F5 (right panels). Dark gray colour corresponds to the menstrual phase; White colour corresponds to the follicular phase; Light gray corresponds to the luteal phase. In signed deviation from pure octave, for pitch B5 (n=9), singers nº 2, 4, 5, and 9 are considered moderate outliers while singer nº7 acts as a severe outlier. For pitch F5 (n=9), singers nº 4 and 5 are considered moderate outliers. In vibrato extent and rate, for pitch B5 (n=7), singers nº 8 and 9 were unable to complete the task. For pitch F5 (n=9), singer nº 5 is considered moderate outlier for vibrato extent and singer nº 6 is considered moderate and severe outliers for vibrato rate for the Placebo and OCP use, respectively.